

Why sequence the human genome?

General enthusiasm for producing a sequence of the nucleotides in the whole of human DNA is well justified, but more attention should be paid to the need for an international data-gathering network.

THE support for ambitions to sequence the human genome provided last week by the US National Research Council (the investigative arm of the national academies of science, medicine and engineering) is predictably intelligent, but may nevertheless help to make an institution of a project that should be much more informally part of the scientific community's endeavour. So much, at least, should be clear from the way in which the committee under Dr Bruce Alberts (see p. 467) considered which of several US government agencies should be the "lead agency" in Washington-speak — and then failed to agree on a solution. The obvious danger is that some inter-agency committee will now take over from where the Alberts committee left off, will bang a few heads together, nominate one or other of the likely candidates and then set about the building of an organization in the mould of the Apollo project.

This is not to say that the sequencing of the human genome is not a worthwhile goal. On the contrary, it is every bit as important as the Alberts committee says. The great savings of effort (and thus of money) that would derive from ready access to a sequence of the nucleotide bases in the human genome are easily understood, perhaps most readily in the continuing search for the genetic basis of inherited disease, but are far from being the chief benefits of a successful sequencing project. It is at least as important that a secure understanding of the function of the apparently redundant DNA in the genomes of higher organisms — is it meaningful, or the consequence of evolutionary history and thus 'junk'? — will not be possible without the complete sequencing of some representative genomes. Another prize, still a long way off, is what will be learned from the inspection of a complete genome about the way in which that genome functions, with sequential and/or selective activation of specific genes. But the Alberts committee is right to draw attention to the importance of comparing the genomes of different species; not only will this advance the identification of functional genes in the sequenced genomes, but the understanding of evolutionary relationships that will also emerge may ultimately be the greatest benefit of efforts now being mounted.

Milestone

Unfortunately, the rhetoric of the past several months has falsely created the impression that the project to sequence the human genome is a self-contained and one-off enterprise — a task with a fixed end-point and thus a restricted (if very large) cost (which is why it appeals to those who manage public budgets and their reception by bodies such as the US Congress). The truth is that even such a specific and substantial task should be regarded as but another milestone, still in the future, in the accumulation of knowledge about the real world. Moreover, it would be folly to suppose that the sequencing business will be worked out, and that molecular biologists can turn their attention to other things, once the sequence of nucleotides in human DNA is neatly tidied away in some computer memory. Those considering what organizations should do the job had better look beyond the immediate goal. That is especially so as there is no shortage of talented people willing to do the job.

What should the public grant-making agencies be doing in the

circumstances? The first need is for support with the development of automatic sequencing techniques, to which government agencies in the United States and Japan have already responded. These initiatives, a necessary preliminary to the human genome project, are nevertheless important in their own right. It will very soon be as pointless that a graduate student's success should be judged by the length of the sequence he presents in the appendix to his thesis as proof of having built a number of amplifiers has long ceased to be a test of competence in nuclear physics. Moreover, the full benefits that will flow in diagnostic medicine from automatic and rapid techniques of sequencing short pieces of the human genome still cannot be guessed at. The \$17 million being spent this year in the United States on these developments will be a sound investment.

Less has been heard of the other urgent need, that for the improvement of the existing databanks of nucleotide sequences, and for the development of novel data-handling techniques. The existing databanks, in the United States and Europe, are already overwhelmed by the present relatively meagre flow of data, much of which is stored without adequate annotation. How would they be able to handle the information that will be available when the even the first instalment of the US investment in automation bears fruit? As things are, there are no arrangements for making sure that the fullest information is wrung from such nucleotide sequences as they find their way to the databanks, but it would plainly be a considerable economy of efforts already expended if the databanks were equipped to answer questions that currently require experimental investigation — to which human chromosome does a notified sequence belong, for example? A further anomaly is that the formal databanks are equipped to handle nucleotide sequences, but that responsibility for maintaining the coarser-scale physical maps of various genomes rests with the academic groups who have made them in the first place. Over this confusing scene is the question of whether, when the sequence of some version of the human genome is successfully stored in a machine, people with a legitimate interest will be able to ask intelligent questions of it.

The moral to be drawn from this state of affairs is that those seeking to create organizations should turn the problem of handling data into an opportunity. If the existing databanks were a necessity when they were first created five years ago, improved versions of them are surely even more necessary now that they are in danger of becoming the victims of their own success. This is where the organization-building should begin. Moreover, as must be plain, the job should be tackled internationally so as to win the greatest benefits from the data gathered in widely scattered laboratories. The ideal would be that such a project should be mounted in a way that commands the respect of the research community. Although the costs would ultimately have to be met by national grant-making agencies, the notion that there might be charges for information supplied should not be excluded. The obvious snag is that the creation of such an organization is bound to take time. But that is merely an argument for starting soon on building an organization without which the human genome project might be a fruitless exercise. □



Can monopolies compete?

British plans to sell the public electricity utility deserve more thought.

IN the middle of a desert, a single ice-cream seller can charge what he likes for his product. The only natural limit is what the market will bear, presumably determined to a close approximation by the cash that potential customers carry in their pockets. This simple prospect seems now to alarm the British government, which plans to sell the nationalized electricity industry to the world at large once it has fought the necessary legislation through the parliamentary session beginning next October. Stung by criticism that the earlier sale of Britain's once-nationalized telephone monopoly has neither improved services nor reduced costs, the government is rightly anxious to create some kind of competition within the privatized electricity industry. In the process, it seems likely to provide a fiery rehearsal of the pros and cons of turning public into private monopolies.

Part of the peculiar British difficulty about this latest scheme for selling public assets is that the British electricity industry is technically as advanced as any other. Only *Electricité de France* has built a comparably integrated network for long-distance transmission — and not even prime minister Jacques Chirac, eager though he is to unwind the French industrial nationalizations of the early 1980s, has dared float the possibility that that might be sold to private shareholders. The strength of both systems is that the output of the generating stations is tightly welded together by a capacious transmission grid, allowing the minimum daily demand for electricity to be generated by the most economical generating stations and making it possible for more expensive generating plant to be used only when needed.

The system works well. In England and Wales, the nationalized generating stations and the transmission grid are operated by the Central Electricity Generating Board (CEGB), which sells its output to a number of regional authorities which, in turn, sell on to individual customers. The system's monopoly has been shaded in recent years to the extent that private persons and companies can now generate their own electricity, although plans for enabling them to sell surplus electricity to the nationalized system have been of little benefit to either party.

How to sell off this system so that the owners of the several parts provide the cheapest service to their customers? Until the end of last week, the government believed it had a solution: sell the area boards to private owners, let them jointly own the transmission grid and leave the CEGB with the ownership of some, but not necessarily all, of the stations now publicly owned. The government seems to have reckoned without the formidable chairman of the CEGB, Lord Marshall, who protested vigorously at the prospect that the management of the power stations might be separated from that of the transmission grid. By all accounts, government plans have been returned to the melting-pot.

The underlying error is a misunderstanding of what constitutes a commercial supply of electricity. What matters to an electricity consumer, and in British circumstances to the area boards, is that there should be a supply of electrons at an agreed potential (volts) and in sufficient numbers (amperes) from a local conductor. It is literally irrelevant how the potential is achieved or where the electrons come from. With the present structure of the British electricity industry, area boards are (or should be) equally unconcerned about the means by which their connections to the transmission grid are provided with current enough to meet their customers' needs at the prearranged voltage, but they are (or should be) concerned on their customers' behalf with the charges they have to pay.

Under the new arrangements, when the components of the industry will be commercial entities, the chief difficulty must be to ensure that enterprises making bad decisions, either about capital investment in new plant or about the instantaneous

operation of the system, should themselves suffer financially and should not be able to avoid the consequences by passing on the costs to their customers. Giving the area boards joint ownership of the transmission grid would allow mismanagement of the system to be concealed. A much better device would be to give the area boards the right to build their own power stations if they chose to do so, thus providing the operators of the transmission grid with a continuing spur to efficiency. By the same test, the new rules should allow for new private entities to build new power stations and even to link them together with transmission grids. That way, the CEGB and its commercial successors would be kept nicely under commercial pressure.

But none of this can constrain the area boards, unavoidably the residual legatees of the public monopoly and potentially the ice-cream salesmen in the desert, from charging what they like for electricity supply. In the sale of British Telecom, the government sought to restrain the upward pressure on prices by crudely requiring that telephone charges should not be increased faster than a fixed percentage less than the rate of inflation during an initial period of five years, but that is a bad precedent. Where telephones and power stations are already owned privately, charges made to ultimate customers are regulated in detail by public commissions. The British government is commendably anxious to avoid the bureaucracy those arrangements entail, but will have to grasp the nettle in the end. The first need is that there should be means of making sure that the charges to individual customers for electricity supply are fair, as between one customer and another or one class of customers and another; this means a more detailed public examination of tariffs than even the public monopoly has had to ensure. Second, there is a need of absolute yardsticks by which profits can be judged to be acceptable. That is where the British government should be concentrating its attention. □

Misguided *perestroika*

Some Soviet engineers feel neglected, and seek an academy of their own. Are they wise?

ONCE upon a time, in the 1930s and even later, Soviet engineers were widely regarded as the salt of the revolutionary earth, but that is no longer self-evidently true. Last week, at a gathering of scientific societies in the Kremlin, one of the questions asked insistently was that of whether the time has come when Soviet engineers should have an academy of their own, to mirror those which already have responsibility for science (including engineering), medicine and agriculture. One argument in favour of the change is that it might enable Soviet engineers to contribute more effectively to Mr Mikhail Gorbachev's *perestroika*. Another, surprisingly, is that engineers at present lack status in the Soviet Union, and deserve more. Both contentions are seductive, but a new academy is not the answer.

It is not as if the strategem has not been tried elsewhere. The US Academy of Engineering and the British Fellowship of Engineering were created to give engineers a "voice", but it is at best a muffled one, while the professional institutions continue to exercise the more decisive influence on education and training. In the Soviet Union, where too many production ministries have too great and too muddled a say in engineering research and development through their own research institutes, there is a more compelling case for change, but to a system in which this hotch-potch of ministry institutes is better managed and more flexibly linked with such needs of as Soviet industry eventually declares. As for the prestige of engineers, there is no reason to suppose that the Soviet need is any different from that elsewhere. And what engenders prestige among engineers (and other professional people) everywhere is that they should enjoy the self-respect that comes from having worthwhile jobs to do, the resources and other requirements necessary for success — and that they should be decently rewarded for their services. □

National Research Council endorses genome project

- Technology development essential
- Initial work can start at once

Washington

IF enthusiastic support from the National Research Council (NRC) counts for anything, the project to map and sequence the human genome has been given quite a boost. In a report released today, a committee of the NRC chaired by Bruce Alberts of the University of California at San Francisco says increased understanding of the human genome "merits a special effort that should be organized and funded specifically for this purpose".

A project to map and sequence the human genome is in some senses already a reality. The Department of Energy has been conducting research at three of its national laboratories aimed at starting the job (see *Nature* 331, 103; 1988), and the National Institutes of Health have received some \$17.3 million specifically for mapping, in addition to the estimated \$200 million it spends annually for genetics research relevant to a genome project. The Howard Hughes Medical Institute is also a substantial contributor to efforts to develop a genetic linkage map, and private companies such as Collaborative Research are also involved.

But a project aimed ultimately at sequencing all 3,000 million base pairs in the human genome has been regarded by most as beyond the resources of any individual company or federal agency. Critics have warned that such a 'big science' biology project would inevitably drain money from other areas of research.

The NRC report, although acknowledging that there are still organizational problems, argues that the scientific merits of the project outweigh its potential liabilities. It suggests that full-scale mapping should begin at once. This would include both genetic linkage mapping — describing the relative positions on different chromosomes of inherited traits — and physical mapping to ascribe absolute positions to cloned DNA fragments.

The report recommends against a large, central facility to begin sequencing the genome, until the technology needed for accurate, high-speed sequencing is improved. Instead, Alberts' panel suggests giving money to individual researchers and medium-sized multidisciplinary groups to achieve the necessary five-tenfold increases in scale and speed of sequencing. Peer review should be used to assess proposals for funding. The panel also suggests a pilot project to sequence 1

million continuous nucleotides, about 10 times more than accomplished so far.

Alberts says the committee is keen to emphasize that although the project's goal is to learn more about the human genome, such an understanding will require continued work on the genetics of other species as well. He points out that yeast cells have many of the same proteins as human cells, but the yeast cells provide a much simpler model for study.

The NRC report says that the genome project will be expensive. The panel estimates \$200 million will be needed annually for 15 years to complete the mapping and sequencing. This money "should not be diverted from the current federal research budget for biomedical sciences", but the report is unclear about what source can be tapped.

Both the federal government and Congress have been trying to decide how the genome project should be coordinated. At present there is an interagency committee chaired by the White House Office of Science and Technology Policy that has oversight responsibility for federal genome activities. But the influence this committee will have is still unclear. The NRC report concludes that a central organizing body will ultimately be needed for coordination.

But the committee could not reach a unanimous conclusion about who should be in charge. Most favour placing responsibility for the project within one federal agency — either the National Institutes of Health, the Department of Energy or the National Science Foundation. Other options considered are an inter-agency committee, or a separate administrative body. But for each of these proposals, Alberts says an independent, influential scientific advisory board is essential.

The initial stage of the genome project should focus primarily on development of appropriate technologies. Alberts says a lot of money for a genome project will allow researchers to take the time to work on initial methodological issues.

Ultimately, of course, the project will develop mountains of genetic data. Although some have worried that these data may be used inappropriately to implement some social agenda, the report concludes that such information will be gathered anyway, and a coordinated and high quality effort will reduce "the chance of misuse of poorly organized information".

Joseph Palca

Move to ban sex determination

Bangalore

THE rural Indian state of Maharashtra has decided to introduce legislation to ban the use of amniocentesis tests strictly for fetal sex determination. This practice, which is becoming prevalent all over India, has led to an alarming increase in abortions. An estimated 78,000 female fetuses were aborted in India between 1978 and 1982, a high figure in part attributable to the increasing number of private clinics that provide amniocentesis services.

According to D. T. Joseph, Secretary of the Maharashtra Public Health Department, the ban will not affect the use of amniocentesis tests to detect genetic disorders such as Down's syndrome and spina bifida. But some doctors do not believe the ban will halt the growing number of amniocentesis-linked abortions, especially in those cultures where producing male progeny is considered essential.

Since 1975, the government of India has banned public hospitals from performing amniocentesis tests for the specific purpose of determining the sex of the fetus, but private clinics do not fall under this legislation. Although it is illegal to have an amniocentesis test performed with the intent of aborting a fetus if it is the 'wrong' sex, nobody has ever been prosecuted for breaking these laws. The cost for an amniocentesis test is about 70–600 rupees (£4–£35), and the cost for an abortion is about the same. The two together, many argue, are much cheaper than the cost of marrying off a daughter.

P. Phatnani, a forensic medicine expert, feels that a ban on amniocentesis tests will not solve this growing problem. He believes that a social awareness of the abuse of the technique is needed. Others feel that such tests should only be allowed in hospitals, and only when there is a family history of genetic birth defects. Medical experts suggest that these tests be carried out exclusively by practitioners specifically licensed to administer the test to ensure there is no abuse.

But many doctors who work in amniocentesis testing centres support the practice, believing that a couple should be able to choose the sex of their children, and that any woman who wants the technique performed will get it done, even by an unqualified person. Others support the belief that by allowing this procedure, they are helping to keep an overflowing population in check, even though recent studies prove that this is not the case. Despite the fact that India has had an adverse sex ratio for several decades now, 935 women to 1,000 men, the population continues to grow.

Radhakrishna Rao

Environmentalist kangaroos for human consumption?

Sydney

MOVES are afoot to change the status of Australia's national symbol, the kangaroo, from vermin to valued commercial resource. According to Professor Gordon Grigg, of the University of Sydney's department of biology, a switch from sheep to kangaroo farming is the only way to halt the desertification of vast tracts of Australia's rangelands in western New South Wales.

Grigg advocates a system where a wildlife inspector would assess the number of kangaroos on a property and issue tags according to the permissible harvest. The kangaroos would be shot on the property, and immediately loaded into refrigerated trucks. Only carcasses with tags attached would be allowed to be processed. Kangaroo meat is compared to venison and is very nutritious, containing only 1 per cent fat compared with the 40 per cent fat ratio of cattle or sheep.

Grigg says the arid rangeland has been severely damaged by the hard hooves of sheep and cattle. "The land is criss-crossed with tracks and beaten to powder", he says, because of overgrazing. As the land becomes more barren, farmers are turning in economic desperation to goats which will eat the woody shrubs left by the sheep. Griggs believes the widespread introduction of goats would be the final ecological blow.

With free-range kangaroos, animals will be able to move to greener pastures in a drought year, with only a sustainable number staying behind. Destruction of

the land would be reversed, according to Grigg, as the kangaroo is a soft-footed grazer eating only the tops of grasses and leaving enough of the plant for it to recover. Seeds eaten by the kangaroo pass undamaged through its system.

Large-scale kangaroo farming has not taken off yet because every state of Australia except South Australia prohibits the



Nice to see the indigenous population being recognized...

sale of kangaroo meat for human consumption.

Australia's influential conservation movement is split over the issue. Some factions are opposed to the economic exploitation of any animal, but others believe that the preservation of habitat takes precedence over manipulation of a single species.

Charles Morgan

No more high-energy physics for NSF?

Washington

APPARENTLY trying to stir up debate, the National Science Foundation (NSF) went to some trouble last week to encourage speculation that the \$42 million it spends each year on high-energy physics might be discontinued. But when questioned, NSF officials played down the idea, and would say only that spending in many areas was being discussed and that high-energy physics was not especially threatened.

In a speech to a joint meeting of the American Physical Society and the American Association of Physics Teachers, Erich Bloch, director of NSF, wondered whether the money that NSF spends on high-energy physics might not more usefully be spent in other ways, leaving high-energy physics entirely in the hands of the Department of Energy (DoE). NSF received only a 3 per cent increase in last year's federal budget, and may now be looking to shed some of its

long-standing programmes to press ahead with new ones, particularly its science and technology centres.

Although NSF's spending in high-energy physics is only 8 per cent of the US total, it supports about one-third of university research. Roy Schwitters of Harvard University, a former adviser to NSF, says that NSF money goes principally to small groups and departments, and supports a wide range of research efficiently and inexpensively. NSF's flexibility gives it a disproportionate influence compared with DoE, much of whose spending is on big programmes.

Bloch has spoken favourably in the past of NSF's role in high-energy physics, and may be airing his thoughts in public to draw physicists to his defence. The less appealing alternative is that unloading high-energy physics is a serious intention, and he wants to gauge the opposition before making moves. David Lindley

Sakharov work acknowledged

London

THE official Soviet news agency TASS has unexpectedly put out on its foreign service an account of the cosmological theories of Dr Andrei Sakharov. Since his return to Moscow from Gor'kii in December 1986, Sakharov has received occasional notices from the Soviet media, but these have been almost entirely connected with his opposition to nuclear weapons and his support for *glasnost*. Virtually nothing has been said of him as a scientist, and the drive to "rejuvenate" the Academy of Sciences made it unlikely that Sakharov, at 66, would return to full-time research.

Nevertheless, in 1980, when Sakharov was exiled to Gor'kii, some Soviet officials said that by removing Sakharov from the proximity of Western journalists and his fellow-dissidents, they were taking him out of the way of temptation, and providing an academic seclusion in which he could return to research. Sakharov's own accounts of his conditions of exile do not bear out this picture but he did produce during these years some papers on cosmological theory which appeared in due course in the *Zhurnal Eksperimental'noi i Teoreticheskoi Fiziki*.

The TASS account deals primarily with Sakharov's latest hypothesis of the existence of a parallel universe of shadow particles that interact only gravitationally whose existence might be demonstrable through observations of black holes. These shadow particles, TASS quotes Sakharov as saying, would be emitted simultaneously with "the particles known to science", during the evaporation of black holes. The rate of evaporation would then be twice that expected if only "known" particles were involved. If a black hole can be discovered, and its rate of evaporation observed, the existence of shadow particles could be established.

This hypothesis, however, is presented as a continuation of the "cycle" of Sakharov's work "of the past decade". (The commentator refers in particular to his 1984 paper postulating that time is multidimensional.) The term "cycle" in this context is significant. For the past few years, State and Lenin prizes for science have been awarded not for individual discoveries but for "cycles" of work over a prolonged period. The implication here is that Sakharov has been working consistently and productively at his cosmology, without interruption. In spite of *glasnost*, official Soviet biographies of "rehabilitated" people still pass in silence over their years in prison or labour camp. And, *glasnost* notwithstanding, Sakharov's years of internal exile seem likely to vanish in the same way.

Vera Rich

Ariane setback makes problems for European space plans

Paris

THE European space industry, which entered 1988 determined to make up for the 16-month setback following problems with its Ariane rocket launchers, is already running late. Despite the successful resumption of Ariane launches in September 1987, the next two flights, V21 and V22, will be delayed. This will make it hard for Arianespace to fulfil its pledge to launch 8 or 9 rockets this year, even though two launch facilities are now available at Kourou in French Guiana.

The next Arianespace launch (V21) has already been postponed once, because of new doubts about Ariane's third-stage rocket motor. But the new delay — until 11 March — is due to a problem in the payload. The power unit of Telecom 1C, one of the two satellites to be launched by V21, has been sent back to its manufacturer, Matra, for emergency revisions.

The satellite's sister, Telecom 1B, stopped transmitting last month when its power supply failed, only 2 years into its expected 7-year life, forcing the French PIT and the Defence Department, its principal users, to switch to the older Telecom 1A satellite. If the mid-March

launch of V21 is to go ahead, the new power supply will have to be delivered to Kourou this week.

Although delays to flight V21 are irritating, Arianespace and the European Space Agency have more reason to be concerned about flight V22. Now scheduled for May 1988, this is to be the first launch of the improved Ariane 4 rocket. Extensive tests on the third-stage cryogenic motor show that the redesigned turbo-pump is registering higher temperatures than specified. It was a turbo-pump failure that stopped V18 in May 1986, only seconds after lift-off, bringing Europe's commercial space industry to an abrupt and costly standstill.

A successful launch of Telecom 1C, even behind schedule, would end a recent run of bad luck with European communications satellites. Not only is Telecom 1B out of action, but West Germany's TV-SAT 1, put into orbit by Arianespace's last launch, still has only one of its two solar panels operational (see *Nature* 331, 200; 1988). Plans to launch TV-SAT's French twin, TDF-1, have now been put back until autumn, and with it West Europe's entry into direct satellite television broadcasting.

Peter Coles

Space station accord

Munich

AFTER years of negotiations, an agreement on the use of the space station Columbus seems very close to being reached by the United States and the European Space Agency (ESA).

It has been difficult to find an acceptable common viewpoint on military use of the space station, to which the European partners are largely opposed, and an equitable way to apportion rights to research results obtained on board the station.

The West German Research and Technology Ministry (BMFT) confirmed reports that significant progress had been made in the latest round of negotiations in Washington last week. A BMFT spokesman said that a memorandum of understanding is expected "in the foreseeable future". West German Research and Technology Minister Heinz Riesenhuber met last week in Washington with US Secretary of State George Shultz. Riesenhuber is currently head of the ESA Council of Ministers.

The spokesman would not comment on reports that the agreement hinged on US capitulation in the area of military research in space. After the details have been settled, it now seems likely that further formal negotiations will not be necessary.

Steven Dickman

New DNA guidelines

San Jose, Costa Rica

THE first guidelines for recombinant DNA genetic engineering work in Latin America have been adopted by a group of Pan-American scientists, in an effort to encourage biotechnology work while providing adequate protection.

Last week's conference of the Inter-American Institute for Cooperation in Agriculture (IICA) was spurred by a decision of Pan-American agriculture ministers in Ottawa last year to encourage biotechnology as a means of revitalizing agriculture in the region. The adoption of the guidelines has also stemmed from fears of "dumping" of uncontrolled genetic engineering experimentation in joint ventures with scientists from more developed countries. Latin Americans are also concerned that agriculture development in the region may suffer if genetic engineering work is not tailored specifically.

The guidelines are based on similar rules already adopted in other countries. They allow regulatory committees to be set up, define the type of experiments requiring approval, classify hazards and put controls on experiments with plants, medicines and foods.

The guidelines will be distributed throughout Latin America with a grant from the Interamerican Development Bank.

Kathy Johnston

No irradiation yet

THE British government's proposals to permit the irradiation of food have been put on ice following intense public opposition. The government accepts the advice of the Advisory Committee on Irradiated and Novel Foods (ACINF) that ionizing radiation can be used safely to improve shelf-life, but concedes that the technique could be used to "clean" unfit food (see *Nature* 328, 751; 1987). In April 1986, two weeks before the Chernobyl incident, the ACINF published a report advising that irradiation was safe. Last week, it published its response to comments received on the original report. It says that of the 6,000 letters received from members of the public and 150 from organizations, "almost all respondents expressed some form of concern about food irradiation".

The report says there is no evidence to support many of the concerns expressed: the formation of toxic radiolytic products, the possibility of free radicals being ingested, or the loss of nutritional value. The government says the process will nevertheless remain outlawed until suitable regulatory controls are devised, including a method to detect whether food has been irradiated.

S. H.

Turks in the Aegean

THE Turkish research ship *Piri Reis*, belonging to the Marine Sciences Institute of the "Ninth September" University of Izmir, is back in the Aegean, this time researching on pollution. Last year, while conducting a geological survey in the same area, the *Piri Reis* was kept under close surveillance by Greek naval vessels, whose tactics, on one occasion at least, alarmed the Turks considerably. This year, according to a Greek government spokesman, Ioannis Roumbatis, there should be no trouble, as there will be no infringement of Hellenic sovereignty so long as the *Piri Reis* stays in the international waters of the Aegean to study pollution.

V. R.

Medical merger off

CURRENCY fluctuations have been blamed for the collapse of a planned joint venture between Britain's General Electric Company (GEC) and Philips, of the Netherlands, to create the world's largest company producing medical diagnostic systems. Last April, the two companies announced the merger of Philips' medical systems division and GEC's Picker International subsidiary (see *Nature* 326, 817; 1987). The new company would have specialized in computed tomography and magnetic resonance systems, with an expected turnover of \$2,000 million. It is believed that disagreement over GEC's investment in the new company, to compensate for its smaller size, led to the deal's eventual failure. It seems likely that both companies will look for other partners.

S. H.

Row over vaccine trial

Washington

THE Argentine ministry of public health is claiming that animal handlers who cared for cows inoculated with a recombinant vaccinia-rabies vaccine have developed antibodies to rabies, suggesting that the virus was shed from the inoculated animals and infected their handlers.

The Argentine government has indicated that it intends to take legal action for redress of damages caused by the way the trial was conducted until it was halted on their instructions.

The experimental vaccine, developed by the Wistar Institute of Philadelphia, Pennsylvania, was being tested by the Pan American Health Organization at its experimental farm in Azul in the province of Buenos Aires. The trial began in July 1986, when 20 cows were inoculated with the virus. The cows were housed with 20 non-inoculated control animals to see if the virus would spread to the controls. But the Argentine government stopped the trial in September, claiming it had not been informed that the trial involved the release of a recombinant vaccine (see *Nature* 324, 202 & 610; 1986). The cows were still allowed to roam through pens enclosed only by barbed wire. The government says that milk from the inoculated cows was sold to local dairies. All 40 animals were killed in November.

According to a ministry of public health report, serum samples from the control animals taken before they were killed indicated the presence of rabies antibodies which had not been present before the experiment. In addition, "some" of the 17 people who had varying degrees of exposure to the inoculated animals developed antibodies to rabies. None of the people has developed health problems, but they will be monitored. A spokeswoman for the Argentine embassy could not say what damages the Argentine government will seek, nor who will be sued.

The Pan American Health Organization has no comment on Argentine claims, saying it is still discussing the case with the Argentine government. Warren Cheston, of the Wistar Institute, says the discovery of rabies antibodies in the animal handlers and control animals is a surprise if it is confirmed. He says serum samples from the control animals available to Wistar researchers taken 3 months after the inoculations did not indicate the presence of rabies antibodies. None of the people exposed to similarly vaccinated animals at the Wistar Institute has ever developed an increased rabies antibody titre. Cheston says a final verdict on the Argentine claims will have to wait until a full release of experimental data is made. **Joseph Palca**

AIDS brochure mailing will go ahead in United States

Washington

Despite a false start last autumn, plans appear on track to mail a brochure containing information on AIDS to every household in the United States. Officials from the US Department of Health and Human Services announced at an international summit of health ministers in London last month that the brochure will be mailed in June or July.

The brochure was supposed to have been mailed last October, as part of the "AIDS Prevention Month" declared by the US Centers for Disease Control (CDC), but political squabbles broke out over the content of the brochure, and which agency should be responsible for it. Although a draft brochure was said to be in the last stages of production, deliberation on the proposed mailing was mysteriously dropped from the agenda of the first public meeting of the presidential commission on the AIDS epidemic. A brochure entitled *What You Should Know About AIDS* was released by the CDC instead, but distribution was limited to local health departments, hospitals and other healthcare practitioners.

The new brochure is being developed by the advertising agency Ogilvy & Mather, and is currently being tested on 'focus groups' of the general population to determine what revisions are necessary to make it most effective. It is likely to focus on correcting the public's knowledge about

the transmission and prevention of AIDS. The level of misinformation about the transmission of the AIDS virus is particularly high. According to data gathered by the National Center for Health Statistics, 36 per cent of the population believes that it is very likely that someone receiving a blood transfusion will be infected with the virus. In addition, 36 per cent feel it is possible to be infected with the AIDS virus by eating in a restaurant where the cook has AIDS, and 35 per cent believe they can contract AIDS from mosquitoes.

Carol Ezzell

Moonlighting OK

London

SCIENTISTS in Liaoning province in north-eastern China are to be allowed to earn at least 50 per cent of their income from spare-time jobs, in an attempt to get them more closely involved with the local economy, the *Guangming Daily* has reported. The provincial authorities have for years been trying to foster links between scientists and local production, the paper said, but the existing contract scheme of payments "limited" the scientists' enthusiasm. In launching the new scheme, the provincial leaders said that any regulations "not conducive to improving productivity or tapping scientists' talents to the full" would be cancelled.

Vera Rich

AIDS research centre to be set up in India

New Delhi

WORRIED about the growing incidence of human immunodeficiency virus (HIV) infection, India has set up a centre dedicated to research on acquired immune deficiency syndrome (AIDS). It will eventually become a fully fledged institute under the Indian Council of Medical Research (ICMR).

The AIDS Research Centre is in Madras, capital of the state of Tamil Nadu, which has most of the 200 HIV-positive cases so far identified in India. One new case is detected every week in the state.

Dr Khorshed Pavri, the retiring director of the National Institute of Virology in Pune, has been made project director of the new centre. A nine-member expert committee has been set up to formulate the centre's research projects. ICMR says the centre will coordinate surveillance and epidemiological work and carry out virological and immunological studies "in the entire spectrum of HIV infection". It will also be the only facility in the country for treatment of AIDS victims.

One research project to be taken up by the centre is on the role of the retrovirus-like intracisternal A-particle (IAP) recently isolated by Pavri from a prostitute with atypical sero-response to HIV. According to Pavri, the IAP, seen for the first time in humans, could act as a precursor to HIV. Interestingly, studies at the ICMR Institute of Leprosy in Agra have shown antigenic cross-reactivity between HIV and mycobacteria that cause leprosy and tuberculosis.

Some ICMR scientists suggest that *Mycobacterium avium intracellulare* (MAI) infection, widely present in India, may be protecting Indians against AIDS in the same way that dengue virus infection protects against yellow fever. ICMR is planning further studies to look for common antigenic patterns and gene sequences between HIV and mycobacteria. Meanwhile, Dr M.G. Deo, director of the Cancer Research Institute in Bombay, has suggested that the cross-reactivity can be exploited to develop an AIDS vaccine containing a number of strains of MAI.

K. S. Jayaraman

Public relations blunders still dog UK nuclear industry

London

THE British nuclear industry's persistent failure to understand the degree of public suspicion surrounding nuclear power generation continues to cost it dear. Last month, the Central Electricity Generating Board (CEGB) planned to demonstrate that the natural circulation of coolant gas in reactors of the Magnox variety is sufficient to cool the reactor after shutdown. The experiment, which would have required authorization from the Nuclear Installations Inspectorate, was to have been carried out at a power station at Trawsfynydd in Wales. The CEGB has performed similar tests in the past without incident and saw no reason to announce its intentions publicly. However, some Trawsfynydd engineers, apparently concerned that the 22-year-old reactor was unsuitable for such an experiment, tipped off the national press. Faced with cries of "Welsh Chernobyl", official protests from the government of the nearby Republic of Ireland and the threatened evacuation of local residents, the CEGB was forced last week to postpone the test. In due course, it says, it will make a "full presentation of what is proposed well in

advance of the test", which might be carried out elsewhere.

Coinciding with last week's announcement to postpone the test was the publication of a document by the Health and Safety Executive (HSE) setting down for the first time the risk targets used by the nuclear industry. John Rimington, HSE's director general, believes, optimistically, that had the document appeared before details of CEGB's experiment were disclosed some of the heat might have been taken out of the debate.

The HSE, which licenses and inspects the nuclear industry, produced the document, *The tolerability of risk from nuclear power stations* (HMSO, £4.95), in response to a recommendation contained in Sir Frank Layfield's report of the public inquiry into proposals to build a pressurized water reactor at Sizewell in Suffolk. Layfield said that the HSE should formulate and publish guidelines on the tolerable levels of individual and social risk to workers and the public from nuclear power stations. The HSE document is intended for public discussion, in line with Layfield's view that "the opinion of the public should underlie the evaluation

of risk . . . there is at present insufficient information to allow understanding of the basis for the regulation of nuclear safety".

The HSE has calculated 'tolerable' and 'acceptable' levels of risk on the basis of those that society and individuals currently accept — such as the existence of petrochemical complexes.

It concludes that for an 'important' release of radioactivity from a nuclear reactor, "a figure that might be accepted as tolerable for a considerable uncontrolled release anywhere in the UK might be about 1 in 10,000 per annum". Such a release could come from a major civil nuclear accident, defined as one giving rise to an uncontrolled release of radioactivity capable of giving doses of 100 millisieverts at 3 km — an accident that might cause the eventual death from cancer of about 100 people. The report says that safety measures in modern reactors reduce the chance of an uncontrolled release to between 1 in 10^5 and 1 in 10^6 per annum. On this basis, a theoretical upper limit of modern nuclear power reactors in Britain would be 100. At present there are 42 operating reactors. **Simon Hadlington**

Further cracks found in superphénix

Paris

DISCOVERY of more cracks in the fuel-rod storage chamber of Superphénix, the French prototype fast-breeder reactor shut down since May 1987, has made a decision about its future unlikely before this autumn. Meanwhile, ultrasound tests to be carried out in May should end speculation that similar cracks have affected the steel casing of the reactor core itself.

In its report to Industry Minister Alain Madelin, Electricité de France (EDF), which runs Superphénix in a consortium with West German and Italian members, said that seven hairline cracks had been located along welded joints in the steel tank that usually contains 600 tonnes of liquid sodium coolant. So far, only one of the cracks — 46 cm long — is known to have penetrated the entire thickness of the steel and is thought to be responsible for the leak of liquid sodium discovered last spring (see *Nature* 328, 100; 1987). EDF and the nuclear industry's security service, SCSIN, have denied claims in French newspapers that all 98 of the tank's welded joints have developed similar faults. Further tests will be carried out this month.

As hopes of repairing the cracked tank recede, the question facing Madelin is whether Superphénix can safely be started up while the storage tank is being replaced. Although not necessary for generating electricity, the tank is at present the only place on site that where fuel rods from the reactor core could safely be placed in an emergency.

Peter Coles

A first for the Japan prize



FOR the first time, a Japanese researcher is among the winners of the Japan prize. Established in 1985, the prize awards ¥50 million (\$390,000) each year to winners in two pre-selected categories. Isao Arita, director of Kumamoto National Hospital, shares the award for preventive medicine with Donald A. Henderson, dean of Johns Hopkins University School of Medicine, and Frank Fenner, formerly of the University of Adelaide (pictured top, left to right), for contributions to the World Health Organisation (WHO)'s eradication of smallpox. Both Arita and Henderson have served as chief medical officer of the WHO World Smallpox Eradication Office in Geneva. Also sharing the prize for preventive medicine, this year split between two quite different areas, are Robert C. Gallo of the National Institutes of Health and Luc Montagnier of the Institut Pasteur (bottom, left and centre), for research on the virus causing AIDS. In the second category, for energy technology, Georges Vendryes (bottom right), scientific adviser to the president of the French Atomic Energy Commission, receives the award for proposing sodium as a coolant in fast-breeder reactors and for his role in encouraging international cooperation in fast-breeder research.

Emigrating Jewish scientists expensive for Soviet Union

London

IOSIF Begun, a former computer scientist and more recently a leading activist for Jewish emigration from the Soviet Union and for the right of Jews remaining in the Soviet Union to learn Hebrew, left Moscow for Israel last month. The media commented that he was the last of the long-term *refusniks* of international renown to leave, and observed that Jewish emigration from the Soviet Union has increased eightfold in the past year.

Such enthusiastic comments are, perhaps, misleading. The International Federation of Scientists for Soviet Refusniks, at a conference in Brussels last November, noted that there were still several hundred Soviet Jews with a scientific background who are denied an exit visa for allegedly having had past access to scientific "secrets". And a month later, an argument dating back to the early 1970s — the "costs" of Jewish emigration to the Soviet Union — was unexpectedly resurrected by *Zviavda*, the party daily of the Byelorussian SSR.

This idea was originally used as the basis

for a demand that Jews wishing to emigrate should repay the cost of their higher education. As education is free in the Soviet Union, the value put on it for this purpose by the authorities was inevitably arbitrary, and then, in 1974, the US Jackson-Vanik amendment (Public Law 504/A) effectively put a stop to the scheme, by automatically excluding from Most Favoured Nation status any government that imposed such a repayment rule.

In December, *Zviavda* claimed that the economic loss to the Soviet Union from the Chernobyl disaster was around 2,000 million rubles. (The Politburo recently put the total cost at 8,000 million rubles.) The paper quoted data from the London School of Economics (LSE) and from a Professor Newland of Washington on the estimated saving to the US economy due to scientific immigrants (\$4 million a year according to the LSE, \$5 million according to Newland) and from the Paris-based bulletin *Les Juifs dans l'URSS* that up to the beginning of the 1980s some 15,000 engineers and 10,000 doctors of medicine

have emigrated to Israel from the Soviet Union, and these, says *Zviavda*, quoting UNESCO, would have cost \$46,000 a head to train, and will earn for their employers some \$250,000 dollars apiece per decade.

These figures are certainly somewhat equivocal. *Zviavda* quotes the total figure for immigration into the United States as if it referred only to Soviet Jews. And Israeli experience of absorbing Soviet Jewish scientists over the past twenty years has not been entirely happy — far too many of the new immigrants have qualified in so narrow a field that it is extremely difficult to integrate them into the Israeli economy.

Nevertheless, the re-emergence of the concept of the "cost" of emigration, even if it has not yet appeared in the All-Union press, is alarming. Under these circumstances, the route followed by the Begun family — to Bucharest and then straight to Tel Aviv — is perhaps significant; for this route has been opened up, as a result of the Gorbachev reforms, during the past year, for those immigrants with a whole-hearted desire to go to Israel, thus bypassing Vienna, the notorious "drop-out" point for those who change their destination for the United States. **Vera Rich**

New Dutch body

Waarle, The Netherlands

ZWO, the Dutch organization responsible since its foundation in 1950 for administering fundamental scientific research, was officially replaced on 1 February by a new body, NWO. The new organization will handle both basic and applied research. The government is no longer represented on the main board. NWO will have five or six science boards, each with responsibility for a separate area. Suggestions for a system of research councils, based on the British model, were rejected. The Netherlands at present spends Dfl 9,100 million (£2,700 million) on research. Of the government's contribution of Dfl 4,000 million, Dfl 1,700 million goes directly to the country's 13 universities as part of the so-called 'first-money flow'. The remaining Dfl 2,400 million will now be spent through the NWO as the 'second-money flow' for applied research, research institutes and participation in international ventures. Part of the second-money flow (Dfl 280 million) will also go into the universities, through NWO. This means that NWO employs around 2,000 of the 10,675 researchers in the universities, with a further 930 researchers in its own 26 institutes.

NWO is continuing ZWO's bilateral agreements with the Royal Society and the Science and Engineering Research Council in Britain, and the French and Italian research councils. **Casper Schuurin**

More woes for New England's beleaguered nuclear power plant

Boston

THE troubled Seabrook nuclear power plant in New Hampshire has received another blow. Its largest owner has filed for bankruptcy protection, the first major US electric utility to take that step since the Depression nearly 60 years ago.

Plagued by its investment of more than \$2,000 million in a plant that has yet to be licensed, and by a 1979 court order that prohibits the company from passing along the added Seabrook costs to consumers until the plant opens, the Public Service Company of New Hampshire was forced to apply for protection to the US Bankruptcy Court in Manchester, New Hampshire, on 28 January.

The licensing process has been long delayed due to the controversy over the implementation of an adequate evacuation plan. The bankruptcy move underlines the malaise afflicting nuclear power plant construction in the United States. No new nuclear power plants have been ordered for a decade, and those ordered since 1974 have been cancelled.

Seabrook's construction costs have risen dramatically. The original estimate of \$1,000 million for two plants at the New Hampshire facility has now climbed to about \$5,000 million for one plant. As a result, Public Service was forced to borrow more and more money at increasingly

higher interest rates.

The profound lack of confidence in the project in the investment community is demonstrated by the inability of the electric utility's bankers to raise \$150 million, despite an offer to pay a full 9 percentage points above prevailing interest rates. The company's common stock, which traded at about \$20 per share in the early 1980s, has plummeted to under \$3.

Initial statements from the utility's management have, not surprisingly, tried to play down the situation, stressing that the bankruptcy should not effect the ultimate licensing of the plant. But critics are already challenging this view. Massachusetts Attorney General James Shannon claims that the bankruptcy strengthens arguments he plans to make against the plant to the Nuclear Regulatory Commission (NRC).

These and other objections were last week reinforced when the NRC rejected the proposed evacuation plan for the Shoreham nuclear power plant in New York.

But supporters of the plant claim that the problem is merely one of extended delays in licensing. Utility spokesman Nicholas Ashooh says: "They force us into bankruptcy because of delays, then argue a license shouldn't be issued because of the bankruptcy." **Seth Shulman**

Government scheme encourages more collaborative research

London

THE British government last week officially launched its much-vaunted LINK initiative, designed to encourage collaborative scientific research between the private and public sectors and to accelerate the commercial exploitation of government-funded research. No new money is being made available for the initiative, which was first announced more than a year ago, and public funding for LINK programmes will be drawn from existing research budgets of government departments and research councils. The government will contribute up to 50 per cent of the costs of a LINK project. Whether LINK will succeed in tempting industry to increase its investment in research remains to be seen.

Research programmes will be in 'strategic' areas of science, for which industrial applications are expected to be attainable with 8–10 years. Proposals for programmes will require approval by a LINK steering group, comprising representatives from industry, the government and academic science. Programmes will usually run for between three and five years, and will be administered by the government department with the greatest financial stake. Thus public support will be withdrawn well before commercial exploitation is in sight, in line with the government's recent declaration that it will no longer fund 'near market' researches.

Five initial programmes have been approved. So far they involve only the Department of Trade and Industry and the Science and Engineering Research Council (SERC) but the intention is that other departments with research interests (notably the Ministry of Defence) will eventually subscribe to LINK. Just over £40 million of government funds is available for the five programmes but will only be spent if matching funds are forthcoming from industry. The eventual total contribution of the government is expected to amount to £210 million over five years.

In general, the initiative has been welcomed by the academic and industrial research community, although there is concern that LINK's long gestation period (it was first announced by Prime Minister Margaret Thatcher in October 1986) suggests underlying difficulties that may not yet be completely resolved.

On the thorny subject of intellectual property rights (IPR), believed to have been one of the main sticking points during early discussions, the government says: "The IPR regime for each LINK programme will be agreed before the programme begins and will be that which the

sponsors and participants consider most suitable for the programme in question." IPR will generally go to the industrial partners "with suitable recompense negotiated for the science base partner(s)". The hope is that disputes over IPR will seldom arise, given that the research will usually be pre-competitive.

A significant concession won by the academic participants in LINK is that money allocated by the University Grants Committee to cover an institution's research overheads will not be considered as part of the government's 50 per cent contribution, and research overheads on a LINK project will be met by the funding agency.

The five approved programmes cover molecular electronics, advanced semiconductor materials, industrial measurement systems, eukaryotic genetic engineering and nanotechnology. No contracts

have yet been drawn up, and most prospective partners have only "expressed interest".

Simon Hadlington

• The first LINK contract is likely to be in the Eukaryotic Genetic Engineering Programme — the smallest of the five programmes. The University of Oxford is close to completing the contract that will establish a Centre for Eukaryotic Molecular Genetics in its biochemistry department with support from British Biotechnology Ltd and Glaxo. A total of £750,000 over four years, half from the two companies and half from the Department of Trade and Industry and SERC, will flow into the centre, where it will be spent on research into the synthesis and secretion of proteins from human cells.

Other projects within the Eukaryotic Genetic Engineering Programme are less advanced, although one centred on the University of Leicester is a strong candidate. But if the £2.3 million available from government sources is to be taken up, further industrial interest will be necessary. SERC is to hold a meeting in March to foster such interest.

Peter Newmark

New US space policy opens up the future to private industry

Washington & Munich

PRESIDENT Ronald Reagan has signed a new comprehensive national space policy that will help direct the future of United States activities in space. The new policy is the result of an 18-month review of previous space efforts, and covers issues of national security, commercial development of space, and research and exploration.

Since 1983, the Reagan administration has been pushing to involve private industry in space services that have traditionally been in the domain of NASA (National Aeronautics and Space Administration). Launch services were the first to be considered, although there was little incentive for private rocket manufacturers to compete with NASA's space shuttle until the Challenger accident put all further shuttle launches on hold (see *Nature* 331, 380; 1988).

Now, private involvement will extend to other areas, including a mini-space station called the Industrial Space Facility (ISF), being developed by Space Industries, Inc. of Houston, Texas. NASA has been concerned that a national commitment to ISF will dilute support for the \$14,600-million space station it is developing. ISF costs are expected to run about 5 per cent of that amount.

But according to a high administration official, ISF will complement NASA's space station, not compete with it. The Reagan administration will ask Congress for \$6,100 million over the next 3 years for

the space station, but only one sixth of that total will be requested for the 1989 budget.

Also included in the new policy is a commitment to NASA's Pathfinder project, to develop advanced technology for the exploitation of space. The administration will request \$100 million in its 1989 budget for this initiative. According to the administration source, the White House feels developing sophisticated technology to send astronauts to the Moon only to abandon it when that task was accomplished was a mistake. The new policy will attempt to provide a more coherent, long-range approach to space that will start in earth orbit, but move beyond as the technology is developed and the need arises. There are no specific goals for renewed human exploration of the Moon or a mission to Mars, but these are certainly possibilities.

The White House now perceives a clear need for a heavy lift vehicle capable of launching payloads weighing between 50 and 100 metric tonnes. This vehicle will go into service in the second half of the next decade, according to current plans.

The president was expected to announce the new space policy in his State of the Union address to Congress on 25 January. However, differences of opinion within the administration over the federal commitment to ISF have prompted an indefinite delay in any formal announcement.

Steven Dickman & Joseph Palca

Prospects revive for Japanese magnetically levitated train

Tokyo

THE advent of high-temperature superconductors has revived interest in Japan in magnetically levitated (Maglev) trains at a time when it may be financially feasible to establish commercial lines.

A few years ago, the prospects for Maglev trains in Japan looked grim. Although a small unmanned vehicle developed by Japan National Railways (JNR) attained a world record speed of 517 km per hour on a 7-km test track in the southern island of Kyushu in 1979, funds for development dried up in the early 1980s as the national railway sank further into chronic debt.

But events over the past year have suddenly made prospects for Maglev much brighter. The break-up and privatization of the national railways freed the newly formed regional Japan Railways (JR) of some of their massive debts, and JNR's laboratories were reborn as a research institute backed by a foundation.

At the same time, the breakthroughs in the development of high-temperature superconductors suddenly made 'superconductor' a household word. And the eyes of politicians and the public turned to the experimental track in Kyushu where a 44-seat prototype vehicle is undergoing tests at speeds up to 400 km per hour. The

fact that JR's Maglev uses conventional niobium-titanium superconductors and that practical wires composed of the new high T_c superconductors are probably decades away (if they are ever developed) has not damped the renewed interest.

Also, the privatization of Nippon



Telegraph and Telephone (NTT) and last year's boom on the Tokyo stock market have brought unexpected millions of millions of yen to the government.

Yet another factor now working in favour of Maglev is foreign competition. Transport Minister Shintaro Ishihara has

just returned from West Germany where he inspected the Transrapid train, a competing magnetically levitated train that uses conventional magnets. There are well-grounded fears here that Transrapid may break into the potentially lucrative US market ahead of Japan. For example, Las Vegas wants to build a 370-km line to Los Angeles to bring in more tourists, and officials from Transrapid say they can meet the city's target for completion in 1995. But Japan has yet to build a test track with curves on it.

Ishihara believes that Japan's Maglev can match Transrapid technologically, but Maglev will not impress the world as long as it is confined to runs "between pig pens and chicken coops" in Kyushu. His ministry has won ¥780 million (\$6 million) in fiscal year 1988 (starting in April) to develop Maglev further and ¥180 million has been assigned to a feasibility study for a track several tens of kilometres in length that could be put to commercial use once tests are completed. Two sites for the test track are under consideration — from Chitose airport to Sapporo, the capital of the northern island of Hokkaido, and from Tokyo to Kofu, the capital of Yamaguchi Prefecture west of Tokyo.

The Maglev train floats on superconducting magnets that induce a repulsive magnet field in aluminium coils set in the floor of the 'U'-shaped trackway while the train is propelled forward by electromagnets in the trackway walls. The train offers a smooth bump-free ride and can climb steeper inclines than a conventional bullet train. Proponents also say that the costs of constructing and maintaining the trackway are less than for an ordinary Shinkansen. But the Maglev train itself costs 4 to 5 times as much as a bullet train.

Prime Minister Noboru Takeshita and many members of the ruling Liberal Democratic party see high-speed trains as a means to de-centralize Tokyo. They also no doubt see it as a way to ensure re-election — it is said that if a politician can have a Shinkansen station placed in his constituency, he is guaranteed a seat in the Diet for life. But resistance from the Ministry of Finance is strong. In December, a ministry official condemned calls for more Shinkansen lines as "one of the three most foolish plans" of this century — the others being the building of the battleship *Yamato* and the construction of the Seikan tunnel between mainland Honshu and Hokkaido. The ministry fears that further expenditures on Shinkansen lines will ruin attempts to bring the former national railway out of debt (much of the debt was accrued through the building of Shinkansen lines). And a Maglev line that short-circuits the present bullet train system would only make matters worse. A decision on future Shinkansen lines has been deferred until this summer.

David Swinbanks

Maglev train system for United States?

Washington

How best to take advantage of the new high-temperature superconductors has been a frequent theme in the US Congress in the past year. Now, Senator Daniel P. Moynihan (Democrat, New York) thinks that he has found a way. Moynihan has introduced legislation to establish a national transportation system of magnetic levitation (Maglev) trains, based upon superconducting magnets. The trains would reach speeds of between 200 and 300 miles per hour, cutting the travel time from Washington, DC to New York City to one hour.

Congress has been worried that the economic fruits of high-temperature superconductivity research may go to other countries despite increased research spending. Exciting results from other countries — notably West Germany and Japan — has convinced many that Congress must help to find ways to exploit the technology and to establish US supremacy in superconductor research. Japan has already completed a prototype Maglev system, with recorded speeds of 400 km per hour (see *Nature* 325, 566; 1987), and Moynihan warns in his legislation that "If

we fail to act now . . . [the project] will be based upon imported foreign technology, either Japanese or German".

Moynihan's bill — the Federal Advanced Superconducting Transportation Act, or FAST Act — would kill two birds with one stone: it puts the new developments in superconducting ceramics to use, and at the same time helps to relieve the nation's air traffic congestion problem. The bill would give NASA (National Aeronautics and Space Administration) \$100 million for research in superconductivity and magnetic levitation over the next three years. It would also establish a High-Speed Ground Transportation Office in the Department of Transportation to oversee the two-year development of the Maglev train system.

Moynihan hopes the system will be under construction within five years after the bill is passed. But critics wonder at his choice of NASA for completing the basic research in superconductivity. The Department of Energy's national laboratories have been leading in the area, and NASA has problems of its own. The current climate of budget belt-tightening also decreases the likelihood of the bill's passage.

Carol Ezzell

The limits of reductionism

SIR—The differences between myself and my friend Steven Weinberg¹ might seem merely to be a semantic argument. Actually they touch upon some of the deepest questions of the Universe and our hopes to solve them. The ambiguous application of the term 'reduction' to a number of different scientific approaches has been the cause of much confusion.

One of the most successful methods of science is that of analysis. It means taking a phenomenon or process of nature and trying to dissect it into its components, and then dissecting again each of these components into its components until the point is reached where one cannot go any further. For instance, after having studied the function of the liver as a whole, I learn a great deal more by studying what goes on in individual liver cells, and finally by determining which molecules play which role in the different organelles of the liver cell etc.

This analytic process is sometimes referred to as *constitutive reductionism* because it consists of the reduction of the studied object into its most basic constituents. No scientist objects to this form of reduction, but it would seem less confusing if such analysis were not referred to as reduction.

Another phenomenon that has been called reduction is *theory reduction*, defined by Nagel² as "the phenomenon of a relatively autonomous theory becoming absorbed by, or reduced to, some other more inclusive theory". The classical example is the reduction of thermodynamics to statistical mechanics. The number of theories that can thus be reduced seems quite limited, and it has not been possible to reduce without residue any biological theory to a physical theory³. Weinberg agrees with this evaluation of theory reduction.

Where the argument begins is when one asks whether the knowledge of the basic constituents of an analysis would permit one to build up an understanding of the total system. We know that an inventory of all the molecules in the liver is not sufficient to reconstruct a description of the function of the entire liver. Without a knowledge of mitochondria and other cellular organelles and structures (membranes), without understanding blood circulation and the structure of capillaries, without knowing what the normal input and output of the liver is, and without a knowledge of many other aspects of the liver and the body as a whole, it would be utterly futile to try to arrive at a correct picture of liver function.

The view that the mere knowledge of its ultimate components would be sufficient to explain a complex system has been referred to by me as *explanatory reduc-*

*tionism*⁴. It has been rejected by many distinguished physicists, including Niels Bohr, and Weinberg agrees with them. Even if you knew everything about every molecule in a glass of water, he says, "how would you ever recognize the properties that interest you about water, properties like vorticity, turbulence, entropy and temperature?"¹. He realizes that "as one goes to the higher and higher levels of organization, new concepts emerge that are needed to understand the behaviour at that level"¹. This acceptance of emergence is quite a departure from the view formerly held by many philosophers and physicists.

Then where do Weinberg and I disagree? Some philosophers have proposed that man in his search for truth deals with three worlds: the microworld of subatomic particles, the middle world from the atom to our Solar System, and the megaworld of the infinite cosmos. Our system of cognition is adapted to deal with the tangible middle world. It is my contention that advances in our understanding of the microworld of subatomic particles are not going to make any explanatory contributions to our understanding of the middle world.

When Weinberg disowned explanatory reductionism, he implicitly agreed with this view. Yet a number of exuberant statements on the last two pages of his article¹ suggest that he expects from the Superconducting Super Collider (SSC) more than a slight advance in our understanding of the subatomic world. He says that "the arrows of explanation point down to a common source" and that in particle physics "we are at a level which is in fact in absolute terms quite deep, perhaps close to the final source", and that the particle physicist finds a "simplicity, a beauty, . . . in the rules that govern matter that mirrors something that is built into the logical structure of the Universe at a very deep level"¹.

I am willing to grant Weinberg that all this might indeed be true as far as the ultimate structure of matter is concerned. I am not a physicist but I would even be willing to accept the claim that some of the findings made in subatomic physics might help to explain certain phenomena of the megaworld. However, Weinberg's stress on the importance of this research implies a hope that it would contribute not only to our understanding of the subatomic world, but also to that of the middle world. I am not so euphoric. Even if the SSC would lead to the discovery of the Higgs boson or some other equivalent finding, my disbelief in explanatory reductionism leads me to doubt that such a discovery would make any contribution whatsoever to our understanding of the

middle world. But it is the middle world that poses all the problems man will have to solve if he is to survive.

I conclude therefore that it is more than doubtful whether the expenditure of 4.4 billion dollars (or more) for the discovery of the Higgs boson can be justified. To be sure, the construction of the SSC is heavily favoured by certain industries and by those politicians who hope that the project will be placed in their state, but whether science as a whole and the United States will derive benefits from this project remains a justified question.

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WEINBERG REPLIES — I can't help but feel that if I could only express myself a little more clearly, Ernst Mayr and I would see that there's really no disagreement between us. So here is one more attempt.

Perhaps the main point that needs clarification is that when I speak of theories as explaining natural phenomena, I am taking for granted a kind of transitivity. If a large number of experimental facts a,b,c, . . . are explained by a set of theories X,Y, . . . , and then these theories are in turn explained by a more satisfying theory Z, then I would say that the facts a,b,c, . . . are explained by theory Z even though we already understood them in terms of the previous theories X,Y, This may I suppose be regarded as just a matter of definition; for me the important thing is that in explaining theories X,Y, . . . , the new theory Z gives us a sense that we better understand why our world (including facts a,b,c, . . .) is the way it is, and that this is one factor that (among other factors) ought to be taken into account in making decisions about the support of scientific research.

To take one example within physics itself: Newton's theories of gravitation and of motion explained a vast number of facts about our Solar System, and about the motion of projectiles and the oceans here on Earth. However, these theories involved several apparently arbitrary elements: the inverse square law, the equality of gravitational and inertial mass, and so on. Also, as realized later, they were in conflict with special relativity. Newton's theories were then explained as an approximate consequence of a more satisfying theory, Einstein's general theory of relativity. In a narrow sense, general relativity contributed very little to our understanding of the Solar System or the tides. We already knew enough to cal-

culate planetary orbits with great precision, and general relativity did not help us with the major puzzles still outstanding (long-term stability, tidal dissipation), only with one tiny anomaly in the orbit of Mercury. Nevertheless, the development of general relativity was important, at least in part, because it explained the theories of Newton that had earlier explained so much else.

Of course, general relativity is important also because it predicts new phenomena, such as black holes and gravitational lenses. This is our historical experience; a theory that provides a more satisfying explanation of what we already knew is likely also to predict things of which we had not yet dreamed.

I suspect that Ernst Mayr would not really disagree with these remarks about general relativity. But in his response to my article he marks out a 'middle world', of scales from the atom to the Solar System, and expresses the doubt that discoveries in elementary particle physics, such as those expected at the SSC accelerator, "would make any contribution whatsoever to our understanding of the middle world". I suppose that he must mean to restrict this remark to *future* discoveries — after all, everyone knows that the discovery of the electron and the atomic nucleus and the quantum mechanical description of their interaction made an enormous contribution to our understanding of matter at the scale of ordinary life. But our quantum theories of electrons and atomic nuclei are clearly not complete — they contain a large number of seemingly arbitrary elements, for instance the fact that the electron is some 2,000 times lighter than the particles in the atomic nucleus. Also, they leave out gravitation.

We are trying to develop a more satisfying theory that explains all these mysteries, a task for which we need new instruments like the SSC. It may be that such a theory would not make life any easier for the fluid dynamicist or the evolutionary biologist, just as general relativity did not help very much in the actual work of celestial mechanics and planetary physics. Yet I think it fair to say that, like general relativity, the sort of theory that we are aiming at in particle physics would, if only by explaining our previous theories, contribute fundamentally to our understanding of the 'middle world'. And if history is any guide, it would predict exciting new phenomena as well.

In the end, we would not be very happy with an understanding of nature that rests on any fundamental distinction between the microworld, middle world and megaworld. We live in one world, and in trying to understand it we discover chains of explanation that cannot be followed farther without looking deeper into the physics of elementary particles. Following these chains down to their roots is not the

only kind of science that is important, but how can anyone doubt that it is important?

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Replacing tenure

SIR—D.A.W. Grant and Bruce Charlton in their letters of protest (*Nature* 328, 754; 1987) about the plight of UK science are both right and both wrong. They are correct to complain about the lack of effective leadership and about the undue acquiescence of bodies such as the Advisory Board for the Research Councils (ABRC) in time- and resource-wasting schemes to give a spurious sense of becoming more efficient by centralizing resources. The shambles created in London medical schools by such an approach should be enough to give pause for thought, before embarking on a much larger exercise based on the same false premise that such reorganizations save money. They do not, and, worse, they are very disruptive. Grant and Charlton are wrong in castigating *Nature* for editorial comments on our plight; we should have listened harder and replied more effectively to both government criticism and *Nature's* prodding. To take a specific point of Grant's, whether the abolition of tenure is good or bad depends on what takes its place. At present, the major advantage of tenure is a practical one: it allows recruitment of staff at lower salaries than would otherwise be the case; even so, we are still losing out to industry and overseas where tenure is much less common. Competitive salaries for (say) rolling 5-year contracts would make us a lot more able to retain or recruit outstanding staff. The main disadvantage of tenure, which is continually hung round our necks and to which we reply so ineffectively, is that it protects the right to idleness (rather than the academic freedom about which we are now hearing such a lot).

The current proposals on tenure will give us the worst of all worlds — new young staff without tenure and existing staff reluctant to move because they will lose tenure; if they do move, it will probably be out of the universities altogether. If the government were to realize the true economic cost of abolishing tenure, it might be persuaded to make more sensible proposals. Tenure should either be abolished for all, with government providing funds to run an economically competitive scheme, or it should be retained (for largely economic reasons) but the universities should be provided with the means and resources to redeploy ineffective staff. Either approach would give us the flexibility to create careers that would be once

again attractive to bright young academics. Our young colleagues' morale is depressed not only by the difficulty of attracting research grants but also by the apparent inability of universities to deal with chronic institutional stasis of life-threatening proportions. To be sure, more money would help, but we are more likely to get it if we show a determined effort to set our own houses in order.

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Creationists now

SIR—With reference to Reginald T. Chelvam's letter (*Nature* 331, 10:1988), our present knowledge is the compound of millennia of accumulation. As prehistoric man pondered on his relationship with the Universe, he encountered the obstacle of the past. The origins of life and the physical world were shrouded in mystery, which was surmounted only by the creation of a 'superhuman' crutch to insecurity. Belief in these various deities has been passed down through the ages. This faith has become so entrenched in man's teachings that many find it hard to accept that the evolutionary theory of the origin of life, and in a wider context matter, has its merits. The more insecure individuals would obviously prefer to cling to the older theory. They are then the centre of the Universe, and life has a purpose and is not the product of aeons of random occurrences.

How can Chelvam equate the narrow-minded creationists of today with the 'default', if you will, creationist founders of our science, who had not even conceived of alternative explanations of origins? Darwinism was never specifically proposed to negate the existence of gods — evolution merely obviated their involvement in the creation of life. There are many (confused?) scientists who believe in evolution and retain a belief in an omniscient, omnipotent being.

We have a long way to go before we understand the Universe, if we ever do. Evolutionary and other theories may be a step on that path, though the teaching of creationism to the next generation as the one and true road will corrupt the attitudes of our future philosophers and scientists. The various theories on the origins of life should be presented to children. They will make up their own minds one day, why not sooner rather than later? Or is it their greater mental freedom that the forces in the Bible Belt fear?

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New gas constant measurement

A new determination of the value of the gas constant, and thus of Boltzmann's constant, seems to be the most accurate in the past fifteen years. But how long will it last?

THE fundamental constants are a mixed bag. Some, such as the unit of time, defined with exquisite accuracy by the frequency of the electronic transitions that make an atomic clock, are uncertain to one part in 10^{10} or thereabouts; if there were a practical need for greater precision, even better standards could be defined without much trouble, perhaps by using the short-period pulsars as the working standards of time. But the precision with which the world in general can be described is necessarily limited by the much less well-defined constants, among which the most conspicuous black sheep is the gravitational constant G , still several parts in 10^5 in error and unlikely to be pursued much more seriously until the question of the reality of the "fifth force" is settled one way or the other.

Not far behind has been Boltzmann's constant or, what comes to the same thing, the 'gas constant' R in the familiar equation of state for a perfect gas, $PV = RT$, uncertain until recently by several parts in 10^5 .

Now a group from the US National Bureau of Standards at Gaithersburg, Maryland and the University of Delaware claims a fivefold improvement of the accuracy of the gas constant by a measurement of the velocity of sound in argon (Moldover, M.R., Trusler, J.P.M., Edwards, T.J., Mehl, J.B. & Davis, R.S. *Phys. Rev. Lett.* **60**, 249; 1988). As a consequence, there is also a reduction of the uncertainty of the Stefan-Boltzmann radiation constant (which depends on the fourth power of the Boltzmann constant) to 6.8 parts in 10^6 . In a field in which people throw their hats up into the air at a factor of two, this is a big step forward.

Inevitably, it is also a reminder of the painfully slow character of progress towards more accurate constants. Each improved measurement seems to entail allowance for systematic errors that were insignificant at earlier stages, with the result that the determination of each constant rests not only on the cleverness of the experimenters but also on a steadily accumulating lore of theoretical calculation.

The principle of the new measurement is neatness itself. Fit a spherical pressure shell with two sonic transducers, one a source of mechanical vibrations of variable frequency and the other a detector of pressure vibrations — a loudspeaker and a

microphone in simple terms. The trick is then to measure the frequency of the input source of sound at which the signal at the receiver is a maximum, which is a mark of when radial sound oscillations within the shell are excited.

Good experimentalists as they are, the authors explain how their measurements have been carried out over a wide range of pressure (up to 500 kPa) and how they have separately recorded five radial oscillation harmonics (at frequencies between 2.5 kHz and 9.5 kHz) at each of a dozen pressures. Measurements under as wide a range of conditions as possible are, of course, the best way of bringing systematic errors to light.

That is why the group itself may have been discontented with one enforced feature of its measurement — the fact that the temperature was fixed throughout to be the temperature of the triple point of water, the condition in which ice, water and water vapour are in equilibrium, and which is not substantially different from the usual melting-point of ice. (Not many of us would answer, if asked, that the scale of temperature is by definition such that the triple point of water has a temperature of 273.16 K exactly.) Plainly the explanation is that the departure from good laboratory practice entailed by not making measurements over a range of temperature is a lesser evil than that of measuring temperature with an accuracy of anything like one part in 10^6 .

Another cause of sheepish uncertainty is the technique for measuring the volume of the 9-cm radius sphere, which has to be done by filling it with mercury from a batch kept at the National Bureau of Standards and used as a convenient standard of density since the early 1960s. The difficulty seems to be uncertainty about the coefficient of thermal expansion of mercury in the temperature range between the triple point of water and the temperature of 20°C at which the mercury was originally standardized.

In its contribution to the final result, this uncertainty ranks as prominently as that of the experimental scatter of the measurements, as is greater than any other single source of error identified by the authors. Understandably, the group looks forward to the use of microwave measurements as an alternative way of estimating the volume of the pressure sphere.

Some of the other sources of error

have been narrowed down in the most meticulous fashion. To allow for residual impurities in the argon used in the measurements, for example, the gases used were compared *in situ* with a sample of ^{40}Ar so as to estimate directly the relative atomic mass of the argon used in the actual measurements.

But much of the error estimation is theoretical in character, and remains unpublished. The authors simply list the results of their calculations, which among other things allow for the way in which the radial sound oscillations may couple to radial oscillations of the pressure shell and, apparently more significant, the thermal boundary layer, expected to be roughly 50 μm thick, arising from the circumstance that energy is dissipated within the pressure shell by the resonant sound waves, and must be conducted away through the argon gas. This effect, in principle, displaces the measured resonance frequencies from the calculated frequencies.

Eventually, readers are promised, there will be a full account of this theoretical basis of the measurement, starting from the Navier-Stokes equations for the behaviour of a compressible fluid such as gaseous argon, and dealing with matters such as the bulk dissipation of energy in the gas and the effects of the thermal boundary layer on the measured frequencies. It appears that the spherical geometry of the new equipment is intrinsically an improvement on the cylindrical systems in which the velocity of sound in argon has previously been determined.

Meanwhile, there seems little prospect that the new equipment will yield a substantially improved value of the gas constant; the authors estimate that "straightforward modifications" would yield only a factor of two reduction of the uncertainty. That is why they pin their hopes on a better technique for measuring the volume of their sphere (on which R is dependent as the $2/3$ power) and the development of sound transducers with a better signal-to-noise ratio at the lower end of the pressure range (where the scatter of the experimental measurements is too great for comfort). Those involved will no doubt be busily at work in the years ahead. Meanwhile, the new value of R , $8.314471 \pm 0.000014 \text{ J mol}^{-1} \text{ K}^{-1}$, has probably come to stay for a while.

John Maddox

Alzheimer's disease

Enter a protease inhibitor

Robin W. Carrell

SCIENCE is an adventure, and never more so than now for those working in the field of protease inhibitors. These inhibitors are popping up in all sorts of unexpected places and their occurrence emphasizes the universal role of proteolysis in the control of biological processes. The most recent finding, reported independently by three groups on pages 525, 528 and 530 of this issue¹⁻³, is of a domain containing a protease-inhibitor sequence, within the precursor of the amyloid β -protein characteristic of Alzheimer's disease.

Whether this inhibitor domain is related to the mechanism of either amyloid deposition or Alzheimer's disease is not known. Certainly there is no evidence of a direct linkage, but there are ample grounds to speculate on possible associations. Amyloid is thought to arise from aberrant cleavage of various proteins to give self-aggregating fragments, with consequent deposition in brain tissues⁴. It is also now known that specific protease inhibitors can act as neurogenic promotion factors⁵. So an aberration of proteolysis must be added to the lengthening list of suggestions as to possible defects underlying Alzheimer's disease.

Current knowledge of Alzheimer's disease, and in particular the role of the amyloid β -protein, has recently been discussed in News and Views articles by Brian Anderton^{6,7}. Two main changes are observed in Alzheimer's brains: one is the presence of intracellular neurofibrillary tangles formed of paired-helical filaments; and the other is the presence of extracellular plaques formed largely by the deposition of amyloid β -protein. These amyloid plaques are closely associated with neurites, the fine dendritic and axonal outgrowths of neuronal cells. The deposited amyloid β -protein consists of a 42-residue peptide, more specifically referred to as the A4 peptide. The A4 peptide is derived from the amyloid precursor protein, a 695-residue glycoprotein which is believed to be a membrane protein with a carboxy-terminal intracellular portion and a large amino-terminal extracellular portion. The A4 peptide, residues 597-638, represents the portion likely to span the membrane-extracellular junction.

The surprise finding of all three groups who report their work in this issue¹⁻³ is the variable presence of a domain of 56 residues interpolated at residue 289, that is, in the proposed extracellular portion of the amyloid precursor protein. This additional sequence shows an identity with the

Kunitz family of protease inhibitors, the best-studied member of which is bovine pancreatic trypsin inhibitor, also called aprotinin. The new amyloid protein sequence is 50 per cent identical to aprotinin and also to the second inhibitory domain of the human plasma protein, inter- α -trypsin inhibitor. This degree of conservation of structure, together with the sequence at the reactive centre (see table), allows a confident prediction that the new domain will function as an active protease inhibitor. Kitaguchi and colleagues³ confirm this by transfection of the cloned complementary DNA of the amyloid precursor protein into cultured COS-1 cells to give an unequivocal increase in tryptic inhibitory activity. The

found to contain functional protease inhibitor domains⁸. A simpler explanation is that the amyloid precursor functions merely as a carrier source for the release of the new inhibitor, in the same way as the other mammalian Kunitz inhibitors, including aprotinin, are known to be released from larger, as yet unidentified, precursor proteins. Both these explanations involve proteolytic processing of the amyloid precursor, an aberration of which could explain the release of the A4 peptide.

A more persuasive possibility, however, is that the new domain has an inherent function as a membrane-bound inhibitor. Cell proliferation and differentiation are known to require a series of surface-related proteolytic events involving serine proteinases with thrombin-like activity. These events are controlled by surface-associated serine protease inhibitors — the protease nexins⁹. One of these nexins has recently been identified as the natural factor that promotes the

	Reactive centre sequences					
	P ₂	P ₁	P ₁ '	P ₂	P ₃	
Amyloid P.P.	-Cys	<u>Arg</u>	-Ala	-Met	-Ile	Kunitz
Inter-α-TI(II)	-Cys	<u>Arg</u>	-Ala	-Phe	-Ile	
Aprotinin	-Cys	<u>Lys</u>	-Ala	-Arg	-Ile	
P. Nexin	-Ala	<u>Arg</u>	-Ser	-Ser	-Pro	Serpins
PAI-1	-Ala	<u>Arg</u>	-Met	-Ala	-Pro	
PAI-2	-Gly	<u>Arg</u>	-Thr	-Gly	-His	
Peptide	-Pro	<u>Arg</u>	-CH ₂ Cl			Synthetic

The table shows the general homology of amyloid precursor protein inhibitor domain with the Kunitz inhibitors (inter- α -trypsin inhibitor domain II and aprotinin). The reactive centre of each inhibitor acts as a putative substrate for its target enzyme which cleaves the P₁-P₁' bond. There is a common active-site P₁ arginine in the amyloid domain and the serpins (protease nexin 1 and plasminogen activator inhibitors PAI 1 and 2) and the synthetic peptide inhibitor (see text). These similarities imply there is overlapping inhibitory activity against serine proteases cleaving at arginyl residues.

same authors also find the variable presence in the amyloid precursor of a further contiguous sequence of 19 residues of unknown homology. Genomic analysis shows that these structures arise from two extra exons, within the amyloid precursor protein gene, which are variably spliced to give three species of messenger RNA. The expression of each of the forms varies from tissue to tissue but in general the proportion of precursor protein with the inhibitory domain is higher in adult than in fetal tissues. Similarly, there is suggestive evidence that the proportion of inhibitor-containing amyloid precursor is greater in frontal cortex from Alzheimer's patients than from normal controls.

What is the explanation for the unexpected occurrence of a protease inhibitor domain in the amyloid precursor? There are several possibilities. It could be there to modify an internal proteolytic event, as is the case with high-molecular-weight kininogen, the source of the vasoactive kinins, which has also recently been

outgrowth of neurites from neuronal cells⁷. This neurite-promoting activity can also be stimulated by other inhibitors such as hirudin and the synthetic peptide D-Phe-Pro-Arg-CH₂Cl. All of these proteins can inhibit serine proteinases that cleave at an arginine, such as thrombin and the plasminogen activators. As shown in the table, these inhibitors all have arginine at the reactive centre (P₁) residue. Thus, the new amyloid Kunitz domain, with a P₁ arginine, is likely to share a similar inhibitory spectrum and thus to be a potential promotor of neurite growth. That this may really be so is

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supported by the earlier histological studies of Probst *et al.*¹⁰ from which they deduce that the amyloid plaque is directly associated with trophic factors that stimulate the abnormal growth of neurites into the plaque.

Taken together, all these observations provide a working hypothesis that plaque formation in Alzheimer's disease is a consequence of proteolysis of the amyloid

precursor protein; self-aggregation of the cleaved A4 peptides explains the precipitated amyloid; and release of a trophic inhibitory domain explains the interwoven neuritic outgrowths. Speculative? True, but it will doubtless be a convincing start for grant applications! □

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Cell biology

Chromosome movement *in vitro*

Roy Burns

THE article by Koshland, Mitchison and Kirschner on page 499 of this issue¹ provides evidence about how chromosomes move towards the spindle poles of the nuclei of cells during the anaphase stage of mitosis. The authors constructed microtubule-chromosome complexes *in vitro* which have the same geometry as one component of the anaphase spindle, and have been able to use them to mimic chromosome movement.

The early anaphase spindle (see figure) consists of two, usually interspersed microtubule systems. One is formed of kinetochore microtubules, running from the two poles to the chromosomal kinetochores: shortening of these kinetochore microtubules results in movement of the chromosomes towards the spindle poles. The second system consists of two sets of interdigitating pole-to-pole microtubules, and continued assembly and sliding in the region of overlap results in the increased separation of the two poles. The first process is called anaphase A, the second anaphase B.

Microtubules have an intrinsic polarity, best defined by their relative rates of assembly at the two ends with the 'plus' end growing about threefold faster than the 'minus' end. All the microtubules in each half-spindle have the same polarity, with the minus ends embedded within the polar centrosomal material, and the plus

ends attached to the kinetochores or lying towards the mid-plane (see figure).

This polarity is central to understanding the microtubule-chromosome complexes constructed by Koshland *et al.*. The authors prepared biotinylated brain microtubules from tubulin, which they then lightly crosslinked and sheared. They used these fragments to nucleate brain tubulin, selecting conditions which restricted assembly to the plus end of the seeds, and then captured isolated chromosomes onto the plus ends of the newly assembled microtubules. The construct therefore resembles the spatial arrangement of the anaphase A system: a microtubule linked to the chromosomal kinetochore by its plus end and with a stable 'cap' at its minus end (see figure).

Microtubule assembly and disassembly is governed by the relative rates of addition and loss of tubulin subunits to the microtubule end. A concentration of free tubulin subunits, the critical concentration, remains when assembly has attained steady state, with pre-assembled microtubules dissociating on dilution until this critical concentration is reached.

Koshland *et al.* diluted their microtubule-chromosome complexes into different concentrations of free tubulin, and then, using immunofluorescence, looked at the position of the chromosomes, the biotinylated stable cap and the inter-

connecting microtubule. They report a remarkable observation: the length of the interconnecting microtubule shortens while remaining attached both to the stable cap and to the chromosome. They go on to show, by using interconnecting microtubules formed in part of partially biotinylated tubulin, that the disassembly occurs at the point of the microtubule-kinetochore attachment. The rate of this disassembly depends, as predicted, on the exogenous tubulin concentration, and can achieve rates of up to $12 \mu\text{m min}^{-1}$.

These *in vitro* results closely mirror *in vivo* observations: for example, photobleaching studies after microinjection of fluorescently labelled tubulin show that the kinetochore microtubules shorten from their plus ends². The *in vitro* experiments do, though, provide one real surprise: the movement of the chromosome along the shortening microtubule does not require either GTP or ATP. This movement towards the minus end does not therefore involve a 'motor', a mechanochemical enzyme such as dynein, kinesin or MAP 1C which effect microtubule sliding or transport of particles along microtubules in various cells (see the recent News and Views article by Ian Gibbons³). Instead, chromosome movement towards the minus end either appears to be driven by energy derived from a conformational change in the disassembling tubulin subunits, or it results from biased diffusion of the kinetochore along the microtubule.

The new results from Kirschner's laboratory provide a further twist. When microtubule-chromosome complexes are diluted into tubulin at concentrations above critical, the distance between the kinetochore and the biotinylated cap remains constant. By contrast, studies in the same laboratory with an earlier model system had shown chromosomal movement at $2.3 \mu\text{m min}^{-1}$ towards the plus end under such conditions, but only when ATP was included⁴. Consequently, a motor is needed for chromosomal movement along a microtubule towards the plus end while disassembly drives it towards the minus end.

Use of microtubule-chromosome constructs is helping to identify the precise molecular basis of one of the most important biological machines. Attention is now likely to focus on the composition and structure of the kinetochore, on how microtubule disassembly induces polewards movement, on the identity of the unidirectional motor, and on the control mechanisms governing the polarity of chromosomal movement. □

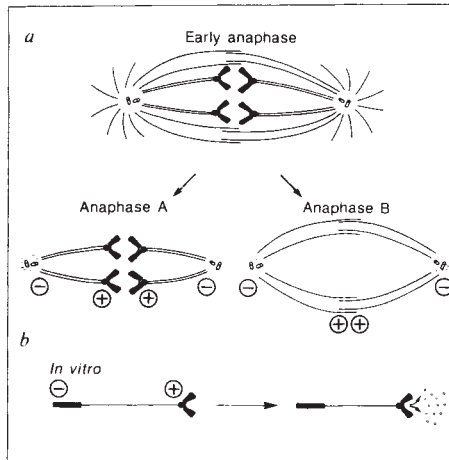
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Orogenesis

Deep geology of the Pyrenees

Andrew M. McCaig

THE publication of a new deep seismic reflection profile is always greeted with enthusiasm. On page 508 of this issue¹, Choukroune *et al.* present the first results of the ECORS profile across the Pyrenees. This is of particular interest because it is the first complete profile across an orogenic belt (mountain belt) and because the deep geology of the Pyrenees has been the subject of intense speculation in recent years. The Pyrenees are especially suitable for detailed study because they were produced by only limited convergence between France and Iberia and because (except in the east) they have not been subjected to later tectonic activity. This means that the whole of the mountain belt is available for study, and (in principle) the complete orogenic history can be deduced.

One of the main objectives in the study of orogenic belts is to define the pre-deformational configuration of crust and mantle, sedimentary basins and earlier structures. In this way, we can hope to elucidate the reasons why structures nucleate and develop in particular geometries, and thus the large-scale rheological controls on crustal deformation. Although several attempts have been made to fulfil this aim by constructing balanced cross-sections across the Pyrenees^{2,3} (in which the conjectured initial geometry is consistent with the known present geometry) there is a wide variety of interpretations because of the lack of data on deep structural geometry in the central part of the chain. The ECORS profile finally allows an objective assessment of this problem, although it will certainly not end the controversy.

It is a tribute as much to the political as to the scientific skills of the ECORS team that the line was ever completed. Cross-border projects are fraught with bureaucratic difficulties, despite the European Economic Community. Even in the present case, the 'continuous' profile is effectively two separate profiles joined together, with only minimal overlap at the international border. The high overall quality of the ECORS line means that the resulting deterioration in the central part of the profile is probably not a serious problem, but it must be a source of annoyance to those involved.

The present-day Pyrenees were formed 30–50 million years ago as a result of limited

(100 ± 20 km) convergence between France and Iberia, while a similar amount of oceanic crust beneath the Bay of Biscay was subducted under the Iberian plate⁴ (Fig. 1). Controversy has centred on three problems: the orientation of thrust faults at depth; the significance of the North Pyrenean Fault (NPF); and the origin of the 'Moho step' (see Fig. 2b) identified by previous seismic work⁵ (the Moho is the boundary between the mantle and the crust). Most authors continue to interpret the NPF as the locus of mid-Cretaceous crustal thinning and sinistral strike-slip motion (the plates slipping leftwards past one other), resulting in low-pressure metamorphism⁶, and the emplacement of slivers of rock from the mantle and lower crust.

The Pyrenees are famous for the fanning geometry of structures produced during Tertiary compression in which faults appear to diverge upwards (see Fig. 2). Many interpretations^{3,7} have emphasized the importance of the NPF as the central feature of this fan, with all thrust faults having a convex-upwards shape rooting as vertical structures in the central part of the chain. This interpretation was strengthened by the observation⁵ of a 10–15-km step in the Moho approximately under the surface trace of the NPF. In the 1980s, however, these ideas were challenged by geologists who favoured a more 'thin-skinned' thrusting model^{2,8} (Fig. 2a). There were objections on both geometrical and rheological grounds to the root-zone model⁹ (Fig. 2b), and it was suggested that the Cretaceous NPF was cut by Tertiary thrust faults in the western part of the chain. In the 'thin-skinned' interpretation, the fanning geometry was the result of backward-

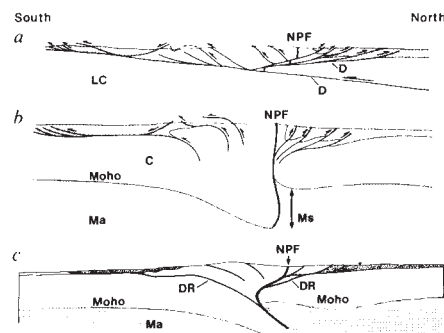


Fig. 2 Models of the origin of the Pyrenees. *a*, 'Thin-skinned' interpretation²: the North Pyrenean Fault (NPF) is cut by shallow thrust faults (décollements, D) below which the lower crust (LC) is not deformed. *b*, Thick-skinned 'root zone' interpretation: the fanning geometry of faults seen at the surface continues to depths. Ms, Moho step; Ma, mantle; C, crust. Large flattening strains are required in the central part of the chain to squeeze out the thrust sheets to the north and south. *c*, Interpretation¹ of the ECORS profile: dipping reflectors (DR) beneath the NPF are interpreted as thrust faults continuous with those seen at the surface. (After the ECORS paper in this issue¹.)

steepening of early-formed shallow thrust faults above north- and south-dipping shallow décollements which cut the NPF. The Moho step beneath the NPF was regarded as a coincidence resulting from approximately equal displacements of the NPF on north- and south-dipping décollements. Deramond *et al.*¹⁰ have put forward a different interpretation; they suggest that the NPF initiated as a shallow, northward-dipping extensional fault that reactivated and steepened during Tertiary thrusting.

How does the ECORS profile affect these hypotheses? Models in which the NPF is continuous and either steep or vertical throughout the crust^{3,5,7,10} now seem to be untenable as two sets of dipping reflectors lie directly beneath its surface trace. Although it is not certain whether these are faults or represent changes of lithology, they can be interpreted as continuations of shallow thrust faults

found in the north and south Pyrenees. Choukroune *et al.*¹ suggest a geometry in which the Iberian lower crust lies essentially undeformed beneath a dominant décollement dipping north at 20°–30°, with higher-level, southward-dipping thrusts cutting the NPF and joining or being deformed by the main décollement (Fig. 2c). This geometry seems most similar to that suggested by Williams and Fischer², although the final dip of both décollements is considerably greater.

Despite the crucial importance of the data obtained by the ECORS team, some caution is appropriate in using

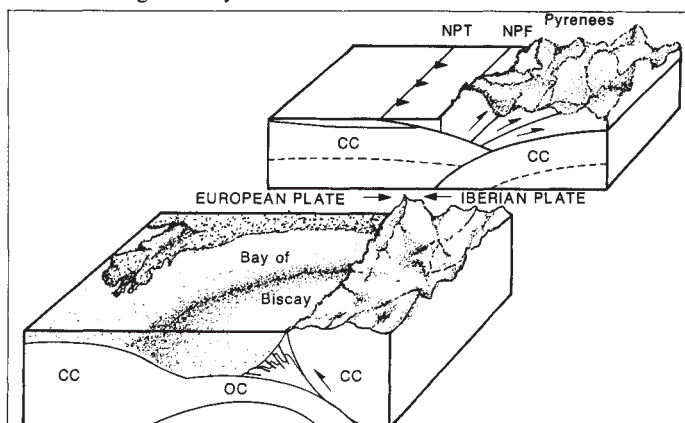


Fig. 1 Slices (not to scale) through the Pyrenees showing (back) the line of the ECORS profile¹ and (front) the subduction of Biscay ocean crust⁴. NPF, North Pyrenean Fault; NPT, North Pyrenean frontal thrust; CC, continental crust; OC, ocean crust.

the profile to interpret the evolution of the whole Pyrenean chain. The relative amounts of displacement on the north and south Pyrenean thrust belts vary greatly along the length of the chain¹, and the ECORS crustal structure may not be typical. The uplift history of the chain must be characterized by further fission-track studies¹¹ (under way at Birkbeck College, London); A. Yelland, personal communication if area-balancing techniques are to be applied to the axial zone. Finally, our recent palaeomagnetic work^{12,13} reveals large rotations of both basement and cover thrust sheets during emplacement. The regional extent of these rotations, together with the tilting history of faults in the central part of the chain, must be determined before properly constrained, balanced

cross-sections can be drawn or the three-dimensional evolution of the thrust system is elucidated. □

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Endothelial mechanosensors

Going with the flow

Jeffrey B. Lansman

ENDOTHELIAL cells form a continuous layer only a single cell thick that lines the entire vascular system. In large and small arteries, these cells respond to acetylcholine and other blood-borne neurohormones by releasing substances causing relaxation of the smooth muscle of the vessel wall (see the recent News and Views article¹ by Paul Vanhoutte). Endothelial cells also respond to mechanical forces generated by blood flowing under pressure. The idea that the endothelium acts as a transducer of haemodynamic forces to control the release of vasoactive substances offers a simple explanation for how local changes in blood pressure or flow lead to changes in vessel tone. Yet how endothelial cells sense fluid forces and couple the initial mechanical deformation of the plasma membrane to the release of vasoactive substances is poorly understood. Olesen, Clapham and Davies have recently reported² the first electrophysiological recording of the response of endothelial cells to controlled levels of fluid shear stress.

These authors reproduced *in vitro* the shear stresses exerted on the endothelium by blood flowing through an artery, in experiments in which they flowed solution over a monolayer of cultured endothelial cells growing on the inner surface of a glass capillary. They find that fluid motion regulates the transmembrane flow of potassium ions (K^+). What must now be answered is whether these K^+ fluxes are involved in any of the many known effects of fluid shear stresses on the biology of endothelial cells and the reactivity of vascular smooth muscle.

Blood flowing through the vascular system stretches and distends the vessels through which it flows. The vessel wall

feels a total force made up of a component perpendicular to it exerted by blood pressure and a tangential component called the wall shear stress, a frictional force produced when a viscous fluid moves across a solid surface. The endothelium feels only a small fraction of the radial stress produced by blood pressure, which is borne mostly by the proteins elastin and collagen in the vessel wall. Wall shear stresses, however, fall entirely on the endothelial layer, so it would not be surprising if endothelial cells possess a mechanism for sensing blood flow.

Shear stresses felt by the endothelial surface vary considerably throughout the vascular system because the characteristics of blood flow depend on vessel size, shape and deformability: steady flow in large arteries produces shear stresses averaging about 2–5 dynes cm^{-2} (a fraction of a millimetre of Hg). In regions where the vessel wall branches or curves sharply, local shear stresses increase 20–50 fold;

when flow becomes turbulent, shear stresses can increase still further³. Pulsatile blood flow causes shear stresses to oscillate, producing added inertial forces. The table lists some effects of controlled shear stresses on endothelial cells.

The K^+ selective current described² by Olesen *et al.* turns on in response to wall shear stresses that fall into the average range of those occurring in the arterial system *in vivo*. On the other hand, the K^+ current saturates at shear stresses well below those produced by altered patterns of blood flow in regions of complex vessel geometry.

An interesting property of the shear stress-activated K^+ current is that it declines during maintained flow. This is reminiscent of the adaptation that occurs to a constant displacement stimulus in mechanoreceptors of certain sensory cells which register dynamic rather than static mechanical forces. That the endothelial K^+ current adapts fits nicely with its role as a flow sensor because blood flow is pulsatile, peaking during each cardiac cycle. It will be interesting to see how the shear stress-activated K^+ current responds to flows that vary with time.

There is good evidence that the endothelium uses information about blood flow to regulate local vessel tone. Large arteries supplying blood to metabolically active tissue dilate so as to accommodate increased blood flows in the tissues. Removing the endothelium abolishes flow-induced vasodilation^{4,5}. The endothelium-dependent vasodilation in response to flow involves the release of a substance causing relaxation. Does the shear stress-activated K^+ current take part in regulating the release of a relaxing factor from endothelial cells?

Olesen *et al.* suggest² that the K^+ current hyperpolarizes the endothelial cell and the underlying vascular muscle to which it is electrically coupled. According to this view, membrane hyperpolarization directly depresses vascular muscle excitability without having an effect on the release of a relaxing factor. If the K^+

Some effects of wall shear stress on endothelial cells

Shear stress (dyne cm^{-2})	Function	Effect	Preparation	Reference
2.8–6.2	Histamine synthesis	Increase	Bovine aorta	13
5–10	Growth	Increase	Bovine aorta	14
	Shape	Elongation in direction of flow	Bovine aorta	14
8	Cytoskeleton	Expression of parallel actin filaments	Bovine aorta	14
10	PGI ₂ secretion	Increase	Human umbilical vein	15
8–12 (1Hz)		Large increase	Human umbilical vein	15
20–500	Arterial permeability	Increase	Excised dog aorta	16
	Attachment to substrate	Detachment		

current helps control the release of a relaxing factor, on the other hand, it might be expected to contribute to a rise in intracellular free Ca^{2+} , which appears to be one stimulus promoting the release of endothelium-derived relaxing factor (EDRF)⁶. How K^+ fluxes or the resulting membrane hyperpolarization might elevate intracellular Ca^{2+} is a problem. Myself and others have found that the aortic endothelium is not electrically excitable and does not have voltage-gated Ca^{2+} channels^{7,8}, so an effect on the activity of Ca^{2+} channels seems unlikely. The possibility remains that shear stresses directly cause the release of membrane-bound Ca^{2+} . Alternatively, the K^+ fluxes may not play a part in regulating vessel tone, but may participate in one or more of the other biological stresses on the endothelium. Good candidates are those responses of the endothelium that show adaptation to maintained flows similar to the shear-stress-activated current.

Changes in vessel tone also occur in response to changes in blood pressure. Elevating the transmural pressure of cerebral arteries, for example, stimulates vasoconstriction. This local response helps keep cerebral blood flow relatively constant over a wide range of arterial perfusion pressure. Again, removing the endothelium prevents the vascular response to a mechanical force⁹. Evidently, stretching the endothelium causes the release of an unidentified peptide which in turn causes contraction of vascular muscle^{10,11}.

Might the endothelial membrane also possess a pressure sensor? Recordings of single-channel activity from the surface of aortic endothelial cells reveal pressure-sensitive ionic channels that allow most cations to pass through¹². Because these channels let Ca^{2+} flow into the cell, an

increase in pressure could stimulate the release of a vasoactive substance directly by stimulating a rise in intracellular Ca^{2+} .

The arterial vascular endothelium seems to have two distinct mechanisms for monitoring local haemodynamic forces that differ in their ionic specificity: a flow sensor that opens K^+ selective channels. It is interesting to speculate that flow-dependent vasodilation and pressure-dependent vasoconstriction are mediated by these two types of mechanosensors. Yet even this simple picture poses many unanswered questions. Most important is how the transmembrane ion fluxes participate in the selective release of relaxing or constricting factors. Elucidation of mechanochemical coupling by the endothelium requires more knowledge of the types of ionic channels and other membrane-bound enzymes that respond to membrane deformation and about the associated changes in intracellular second messengers. □

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Plant ecology

Quarrying for extinct species

Peter D. Moore

THE discovery of viable seeds of rare plants in the soils of sites where they had previously been considered extinct brings into question the definition of extinction for the plant kingdom¹. In the fenlands of East Anglia in Britain, the fen ragwort (*Senecio paludosus*) and the fen violet (*Viola persicifolia*) have put in an unexpected appearance at two sites after many decades of absence. One likely explanation is that the seeds have germinated from a long-dormant seed bank. If this is true, should conservationists be concerned with the assessment not only of the current vegetation of a potential nature reserve, but also of its soil seed bank? This question has now been investigated by Jefferson and Usher² in experiments in

chalk quarries in northern England.

Jefferson and Usher selected three chalk quarries that are rated as rich and important in terms of plant conservation. Their choice of a habitat that is essentially man-made and that has suffered considerable disturbance historically is significant, for most soil seed banks are dominated by species of early, open habitats, so such plants must have existed in the quarries in relatively recent times. Indeed, some of the richest and most interesting areas of the quarries at the present time are the open floors, where disturbance has persisted longest. In the areas where succession has been allowed to proceed undisturbed for a long time, a tall grassland develops that is relatively poor in plant species and

in which many of the smaller plants and those with shorter generation times have become 'extinct'.

Jefferson and Usher took soil cores from two successional stages at 6-week intervals over a year's study and germinated the resident population of seeds within them. They find that seeds are present and viable in the soil but that they are not represented in the plant community from which the samples were collected. Of the three quarries studied, the number of species that germinated in the samples but were absent in the quarry sites varied from two (5 per cent of seedling taxa) to nine (20 per cent). Most of these species are not rare plants but one, the small toadflax (*Chaenorhinum minus*), is rather local in northern England.

One possible explanation of these results is that some of the seeds germinating from soil samples have entered the community by dispersal from outside³. This event is particularly likely for some species, such as *Sagina apetala*, with its dust-like seeds, and *Epilobium hirsutum*, the seeds of which bear a plume of long hairs and are easily transported by wind. This latter species is also ecologically an unlikely candidate for having grown in a dry chalk quarry. Recent studies⁴ of seed dispersal in a wind-transported composite, the ragwort (*Senecio jacobaea*), show that even in this efficiently dispersed species 31 per cent of fruits travel only 1 metre and 89 per cent have fallen from the air within 5 metres. So it is probable that most of the seeds found in quarry soils were produced very locally, even those adapted for wind dispersal, and therefore that they did not come into the area from outside.

It is evident that species present in the soil seed bank yet absent in the vegetation cannot be regarded as truly extinct at the site, and that disturbance of the soil could regenerate these lost species. Jefferson and Usher tested this supposition by scraping sample areas of quarry soil and observing the consequent germination. The floral mix resulting from such experiments most closely resembles the floristically rich, quarry-floor assemblages in their species composition, showing that diverse quarry floras can indeed be re-created by scraping off old-rank grassland.

Perhaps the greatest problem facing the quarry conservationist is persuading well-meaning members of the public that ripping off some of the grassland from over-mature quarries is not an act of flagrant vandalism, but a means of releasing long-lost species from decades of dormancy. □

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Invertebrate neuroendocrinology

Insulin found at last?

Alan Thorpe and Hanne Duve

THE discovery of an insulin-related neuro-peptide in the cerebral ganglia of the mollusc *Lymnaea stagnalis* reported by Smit *et al.* on page 535 of this issue¹ provides convincing evidence that functionally important peptide structures have been conserved through a very long period of evolution. Such a basic concept has not always been readily accepted in the field of neuroendocrinology, where mammalian (or at least, vertebrate) studies have dominated. Thus, insulin and other important regulatory peptides have been considered by some to belong uniquely to the vertebrates, having evolved there in response to particular metabolic and physiological requirements.

Previous studies on invertebrates suggested that insulin-related peptides are present in a range of different species (notably insects and molluscs²). The evidence for this has relied largely on the use of mammalian insulin antisera in cytological and purification studies (immunocytochemistry and radioimmunoassay). However, there were no confirmatory amino-acid sequence data of an insulin-related structure in invertebrates until the recent important work of Nagasawa and his colleagues³ on the prothoracicotrophic hormone (PTTH) of the silkworm *Bombyx mori*. This neuropeptide, which is produced by the median neurosecretory cells of the pars intercerebralis of the brain, controls the secretion of ecdysone from the prothoracic glands during metamorphosis. Although PTTH was not previously suspected of having any relationship to insulin, Nagasawa *et al.* purified it from more than half a million heads of *Bombyx* and established that one form of the peptide (4K-PTTH-II) consists of two non-identical chains with, respectively, a 47 and 30 per cent homology to the A and B chains of the human insulin molecule (see figure). Perhaps of even greater significance, the PTTH molecule can assume an insulin-like tertiary structure on account of the conservation of most of the hydrophobic core residues.

The sequence data on the insulin-related peptide of *Lymnaea* corresponds well with that from *Bombyx*. It, too, shows the presence of cysteines at positions in the A and B chains typical for the insulin family, and it also suggests the formation of an insulin-like tertiary structure with conserved (or alternative) hydrophobic core residues (see figure). In addition to the timely and important information on a second invertebrate insulin structure, the molluscan study of Smit *et al.*¹, by using the recombinant

DNA approach, provides the first evidence of an invertebrate preproinsulin structure, complete with a C-peptide equivalent and a putative signal sequence.

The finding of invertebrate insulin-like molecules highlights several important issues. First, the resolution of these molecular structures leaves little doubt that the insulin family of peptides has had a long evolutionary history, dating back to the pre-vertebrate era. Members of the group must now include not only vertebrate representatives such as the insulins themselves, the insulin-like growth factors

significant advantage have been conferred by extra-neural sites of production such as the gut, pancreas and other endocrine glands. In a discussion on the neural origins of peptides it is relevant that although the particular question of the brain insulin of vertebrates is somewhat controversial, especially in relation to the actual sites of synthesis⁷, nevertheless insulin receptors are present in the brain where they are associated with specific insulin-mediated functions⁸.

The many roles of peptides of the insulin family include a growth-promoting activity that is exerted maximally by IGF-I and II but is also shown in varying degrees by insulin molecules *per se*⁹. In this respect, it is of interest that the molluscan insulin-like peptide is the product of cells noted for their role in the control of growth. Structure/function relationship

A Chain	1	5	10	15	20		
Human	- - - -	G(I)V E Q C(C) T S I C(S) L Y Q L E N V(C) N					
<u>Lymnaea</u>	Q G I T N	I(V) C(F) C(M) K P(C) T L S E I R Q V(C) P					
<u>Bombyx</u>	- - - -	G(I)V D E C(C) L R P(C) S V D V L S V(C) -					
B Chain	1	5	10	15	20	25	30
Human	- - - - -	F V N Q H L C(C) S H L V E A L Y L V C(G) E R G F F Y T P L T					
<u>Lymnaea</u>	Q F S A C N I N D R P H R R G V C(C) S A L A D I L V D F A C S S S N Q P A N V - -						
<u>Bombyx</u>	- - - - -	E Q P Q A V H T T C(C) R H L A R T L A D I C W E A G V D - - - -					

Amino acid sequences of Lymnaea and Bombyx insulin-related peptides compared with human insulin. Homologous residues indicated by circles (see refs 1, 3).

Amino acid sequences of *Lymnaea* and *Bombyx* insulin-related peptides compared with human insulin. Homologous residues indicated by circles (see refs 1, 3).

(IGF) I and II and relaxin^{4,5}, but also invertebrate peptides such as PTTH and, now, the *Lymnaea* insulin-related peptide. Second, it can be assumed that at least some of the many published immunological data on the insulin-like peptides of other invertebrates² and also unicellular organisms⁶ have a basis in true molecular interactions between mammalian insulin antisera and cellular antigens with an insulin-like character, rather than in non-specific cross-reactions. Characterization studies of these peptides are required to provide a more complete evaluation of the evolutionary history of the insulin gene(s).

The localization of invertebrate insulins within specific neurons of the brain suggests that the nervous system of early multicellular organisms was, indeed, the original source of these and perhaps other regulatory peptides that are found in present-day vertebrates in various sites in the body, including the nervous system (the so-called brain/gut peptides). These biologically active molecules can be assumed to have exerted their controlling influence over metabolic or physiological functions within the body as a whole, in the manner of the modern-day invertebrates, following their release from neurosecretory axons terminating on neurohaemal areas. Only later, when large body size became a limiting factor to the distribution of the active peptides, would a

studies on a large variety of insulin-related peptides and analogues suggest that the growth activity of insulin and IGFs involves a molecular domain (as yet unidentified) that differs from the receptor-binding region of insulin involved in metabolic activity⁸. Invertebrate insulins known to have specific growth-promoting roles could be very useful in the analysis of this interesting problem.

Finally, no insulin molecule is complete without its receptor, and although something of the nature of invertebrate insulins is being elucidated, we have little knowledge of binding sites or cellular mechanisms within these animals. Insulin receptors with certain characteristics similar to those known from mammalian studies (affinity, specificity, size and enzymatic activity) have recently been

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demonstrated¹⁰ in the fruitfly *Drosophila*. This receptor is not, however, recognized by anti-receptor antisera directed against insulin receptors of human placenta or rat liver, a feature that suggests structural differences between the insect and mammalian receptors. In regard to studies of this type, it is relevant to note that the considerable variation in the peptides of the insulin family that exists presumably as a result of evolutionary selection pressures, is likely to be matched by an equal variation of the receptors. As an example of this, the structural homologies shared by insulin and IGF peptides are paralleled by

a structural homology of the receptors for insulin and IGF-1 (ref. 11).

Insulin is, perhaps, the most actively researched of all biological molecules and the variety and number of problems associated with it seem to increase exponentially with every new advance that is made. The realization that invertebrates as well as vertebrates are custodians of this molecular species adds a further challenging dimension to the problem of insulin. □

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Oceanography

Stirrings in the abyss

Nick McCave

THE notion that deep waters run steadily, if not still, receives another knock on the head from the work of Gross *et al.* on abyssal storms in the North Atlantic reported on page 518 of this issue¹. It was the prevailing view 15 years ago, most eloquently put by Heezen and Hollister², that "the warm water sphere protects and isolates the abyss from the more familiar forces of weather, leaving... the thermohaline currents which spread and mix from pole to pole, the rotation of the earth which deflects these currents to the side of the basin and the tides which gently, but incessantly, caress the abyssal waters". Not that this steady thermohaline flow was everywhere seen as gentle, for several places, particularly straits and passages, displayed striking photographic evidence of current scour² (see figure). Gross and co-workers document with detailed velocity, turbulence and turbidity measurements one of several significant perturbations of the mean flow called deep-sea storms³.

Gross *et al.* worked in the high-energy benthic boundary-layer experiment (HEBBLE), which was designed to examine interactions between deep flows and sediment beds in a region expected to be dominated by steady, swift currents towards the south-west. Although the authors did indeed find swift currents, they were markedly unsteady. The work of several physical oceanographers compiled by Schmitz⁴ and Weatherley⁵ shows that the eddy kinetic energy (K_E , variance of the current velocity) is high at depths greater than 4,000 m in part of the western North Atlantic. The zone of high abyssal K_E appears to underlie directly the region of

highest surface K_E found in the Gulf Stream. This led to the suggestion that the episodes of high-speed bottom currents which stir up the bed ('storms') are related to downwards propagation of eddy energy from the upper ocean^{4,5}.

Recent current-meter results from the highly energetic east Australian Tasman Sea region also show high-speed bottom currents related to the passage of surface warm-core rings, and sediment bedforms,

uous record of turbulence and turbidity for a year at five levels above the bed in the deep ocean 5 km down. Such a record has not ever been obtained, even in shallow water. It will be instructive to compare it with other records of planetary boundary layers on land, over the sea and under ice. Records of the deep sea were somewhat easier to obtain because there is no contamination by surface waves, thus permitting *in situ* data analysis and compression. Only the time-averaged components of the Reynolds stress are stored rather than the four separate data sets accumulated at 2 Hz by each current sensor. The storm Gross *et al.* report with a maximum speed of 22 cm s⁻¹ is relatively modest (although earlier reports¹¹ of speeds up to 74 cm s⁻¹ are in error by a factor of two), but it produces a very high turbidity. Thus, reported turbidities of up to 12,000 mg m⁻³ are plausibly accounted for by bottom-flow speeds up to 40 cm s⁻¹. Note that the critical erosion speeds at the onset of the storm implicit in Fig. 2 of Gross *et al.* on page 520 are 14 cm s⁻¹ (4.5 m above bottom) or a shear velocity of 0.68 cm s⁻¹.

There are many areas of the deep sea where speeds in excess of 14 cm s⁻¹ are achieved at least intermittently. The importance of this observation lies in the uses that can be made of the deep-sea bed.

In waste disposal, for example, it emerges that there are some areas from which particulate materials will be raised up and dispersed far and wide, whereas other areas will be more retentive. For the installation of listening devices on the sea bed, the results warn which areas would alternately scour and blanket them with deposits of mud. There may even be implications for the routing of deep-sea cables: turbidity currents, slumps and debris flows have normally been seen as their principal enemies, but it now seems to be possible that some stormy areas of the abyss could be worth giving a miss. □

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

The effect of a deep-sea storm in the North Atlantic. Left, taken during a storm on 29 November 1985. Small features have been hydraulically smoothed by the flow. Five days later (right) the flow has slowed and the bed is roughened on the smallest scale (1–2 cm) by animal tracking, but is smoothed on larger scales by the moderate flow from the north-west. (Courtesy T.F. Gross and A.J. Williams III).

both indicating (temporary) strong flow counter to that expected for the regional thermohaline flow^{6,7}. These observations and those of HEBBLE indicate that satellite and drift maps of surface eddy kinetic energy^{8,9} will be a useful guide for searches for energetic, eroding bottom flows. The most distinctive region in this respect is the circumpolar Southern Ocean, which may thus be not only the 'lung' of the oceans but also the source of most turbid bottom water¹⁰.

The achievements of Gross *et al.* reported in this issue¹ and previously are noteworthy in terms of instrumentation. The authors have obtained a near contin-

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Evolutionary biology

What do we know about speciation?

J.A. Coyne and N.H. Barton

ALTHOUGH darwinism is often declared to be dead, it refuses to lie down. Darwin did, however, mislead his audience in one way: his best-known work is much more about the origins of adaptations than of species. Since then, there has been much more progress in understanding the causes of adaptive change than of the mechanisms whereby new species are generated. In the past decade, attention has shifted back to the problem of how and why living creatures are organized into clusters of similar phenotypes. A perspective on this long-standing puzzle was provided at a recent meeting*.

Clustering can arise in several ways: interbreeding among sexually reproducing organisms; descent from a common ancestor; constraints on available genetic variation; or direct adaptation to environmental factors. These different pressures are reflected by diverse definitions of 'species'. The most widely accepted is the 'biological' species: a group of individuals characterized by heritable differences that prevent exchange of genes with related groups¹. A.R. Templeton (Washington University, St Louis) argued that species definitions should instead be in terms of the processes which maintain the integrity of a cluster: he suggested defining "a group . . . whose range of variation is limited by genetically based cohesion mechanisms". As an alternative, J. Cracraft (University of Illinois, Chicago) put forward a 'phylogenetic' species concept, in which a species must include all individuals that share a common ancestor. All these definitions coincide where distinct groups coexist without interbreeding. But each is at least as difficult to apply to populations in different places as the traditional concept of the biological species. Both may split biological species into arbitrarily small units: taxonomy is hard enough without admitting this possibility.

What is the relation between reproductive isolation and morphological clustering? Although the two must usually be correlated, there is little support for the tenet of punctuated equilibrium, that substantial morphological change is permitted only by the simultaneous establishment of reproductive isolation². Fully isolated species can be osteologically identical, so that speciation events may frequently be missed in the fossil record (A. Larson, Washington University, St Louis). Conversely, immense morpho-

logical change can occur without noticeable reproductive isolation, as in the allopatric races of the Hawaiian plant *Bidens* (F. Ganders, University of British Columbia). Although speciation is not required for morphological change, it can preserve it, because reproductive isolation can perpetuate local adaptations that would normally disappear with ecological change and gene flow (D. Futuyma, State University of New York, Stony Brook). This might yield an apparent correlation between speciation and punctuated morphological change.

There is still no disagreement with the

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Did the Hawaiian Drosophila evolve through founder events, sexual selection or adaptive divergence? Left, D.heteroneura; right, D. nigribasis. (Courtesy of M.P. Kambyzellis.)

dogma that speciation in sexual groups usually requires geographical isolation but occasional sympatric speciation (that is, speciation within one area) has not been excluded. A first step in this process, the development of polymorphism for resource use in a single population, was described for bluegill sunfish by D.S. Wilson (Kellogg Biological Station, Michigan) and for Galapagos finches by P. and B. Grant (Princeton University). It is also known in *Pyrenestes* finches from Africa³. The genetic basis of all these polymorphisms is unknown, however, and a crucial step — the evolution of assortative mating — has not occurred in the finches and is not yet known in sunfish. Partitioning of niches within a species is probably far more common than is sympatric speciation; indeed, the polymorphism of Galapagos finches completely disappeared with an ecological change on the island.

The factors that hinder sympatric speciation also cause problems for the evolution of reinforcement, the evolutionary increase of mating isolation which might occur when partially intersterile populations come into contact. This once popular concept lacks theoretical and empirical support: several other processes can

produce heightened mate discrimination where species overlap, and evidence from narrow hybrid zones suggests that reinforcement is rare (R. Butlin, University College Cardiff). However, the observation that sympatric pairs of *Drosophila* species show much stronger assortative mating than do allopatric pairs of similar age is strong evidence of reinforcement (F. Ayala, University of California, Irvine; J.A. and A. Orr, University of Chicago). Theoretical models reveal many obstacles to reinforcement, but do not exclude it⁴.

Hybrid zones can also reveal the ecological and demographic factors which influence interactions between divergent populations. Even within a group, hybrid zones can range from smooth intergradations with extensive hybridization to narrow overlaps containing few hybrids (for example, *Ensatina* salamanders, D. Wake; *Thomomys* pocket gophers, J. Patton and M. Smith, all at the University of California, Berkeley). In the alpine grasshopper *Podisma pedestris*, two chromosome races meet along a mountain ridge, and apparent anomalies in gene position can be accounted for by local density⁵ (G. Hewitt, University of East Anglia). In contrast, the hybridizing crickets *Gryllus firmus* and *G. pennsylvanicus* are distributed in a mosaic across the eastern United States, in direct response to local soil type⁶ (R.G. Harrison, Cornell University, New York).

Ecology and demography are also relevant to another area of current controversy: the importance of founder events in speciation. Much work has been stimulated by the dramatic radiation of the Hawaiian *Drosophila* (see figure), in which more than 800 species have evolved in the relatively short time since the archipelago was formed (K. Kaneshiro, University of Hawaii). Early theories stressed the importance of loss of genetic variability⁷. However, arguments that population bottlenecks would not by themselves cause strong isolation (N.H. B.), and the striking sexual dimorphism in these flies, have led to the suggestion that founder events trigger runaway coevolution of male traits with female preferences⁸ (Kaneshiro). Other equally dramatic island radiations have, however, prompted straightforward adaptive explanations. D. Otte (Academy of Natural Sciences, Philadelphia) argued that the extremely diverse songs of Hawaiian tree crickets have evolved primarily by selection against overlap with neighbouring species. On the Galapagos, the selective pressures which have forced Darwin's finches into diverse feeding strategies can be identified (Grant and Grant). The problem is to disentangle the effects of population bottlenecks, sexual selection, and adaptation to novel physical and biotic circumstances in causing speciation — a task that may be

* Speciation. Academy of Natural Sciences, Philadelphia, 5-8 November 1987.

insuperable.

Our understanding of speciation has progressed depressingly little since the classic reviews by Dobzhansky¹ and Mayr⁹. This is partly because of the difficulties of studying a historical process. But it also results from the preoccupation with schemes such as sympatric and chromosomal speciation, and speciation caused by genetic drift, that have little theoretical or empirical support and are considered infrequent even by their proponents. Like Wright's shifting balance theory, these processes may operate in nature and may even be demonstrable in the laboratory, but in nature are hard to distinguish from better-established alternatives.

Nevertheless, there are some questions that seem more tractable. How many genetic changes separate two species? Although there are straightforward methods for mapping genes causing reproductive or morphological differences, there have been comparatively few genetic analyses of closely related species. In most animals, reproductive isolation stems from changes at many genes, but speciation in plants may often involve very few loci¹⁰. Are there other differences between plant and animal speciation? The Hawaiian *Bidens* show that substantial adaptive divergence can occur without reproductive isolation, perhaps because of the compartmentalized development of plants. What is the normal function of genes that cause reproductive isolation? Mapping should soon permit cloning and sequencing, and provide clues to their

normal role in development. More attention should be devoted to the possibility of speciation through sexual selection, where the connection between selection and reproductive isolation is clear. Are there 'rules' of speciation that apply across groups? The ubiquity of Haldane's rule in animals (the preferential sterility of heterogametic hybrids) and its likely origin by rapid evolution of the sex chromosomes¹¹ are two patterns without widely accepted evolutionary explanations. Comparative studies may reveal other such patterns and underscore the need for good systematics and accurate estimates of divergence times. A combination of genetic and developmental approaches may offer the most progress in understanding the evolution of reproductive isolation. Without such knowledge, we are simply unable to evaluate the many theories of speciation. □

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Vaccine development

Which way for malaria?

F.E.G. Cox

THE discovery, recently reported¹ by Louis Schofield and collaborators, that immunity against malaria operates against stages of the parasite developing in the liver calls for a radical reappraisal of the ways in which malaria vaccines are being developed, and at the same time explains several paradoxes.

The life-cycle of the malaria parasite begins when a mosquito injects infective stages, called sporozoites, directly into a blood capillary. Sporozoites enter liver hepatocytes within minutes and undergo a massive phase of asexual multiplication that results in the formation and release of up to 30,000 merozoites. These invade red blood cells and undergo further cycles of multiplication, destroying increasing numbers of erythrocytes. Sporozoites, liver-stages, merozoites and intraerythrocytic stages of the malaria parasite possess largely unique repertoires of antigens and, although clinical immunity is associated with the blood stages, the sporozoite is the

obvious target for a vaccine.

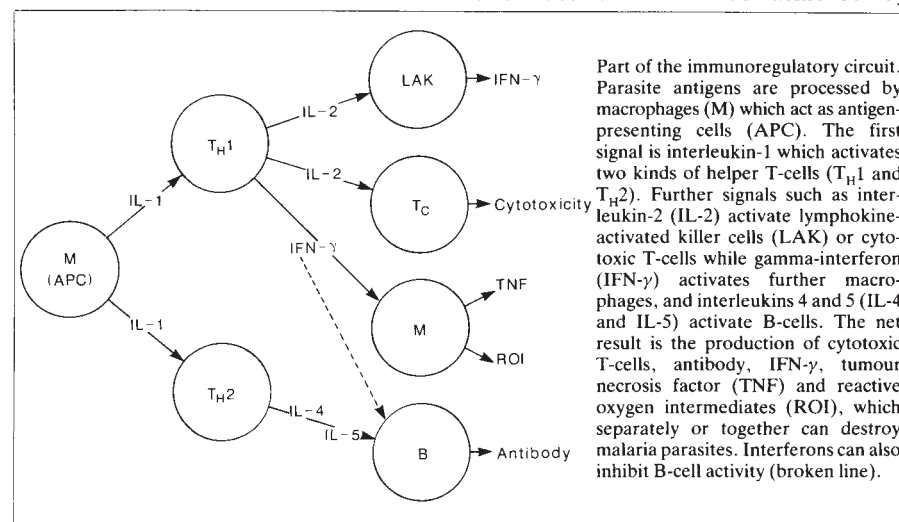
Attempts to develop vaccines against sporozoites and blood stages have concentrated on the identification and

synthesis of dominant surface proteins², but despite showing initial promise, this approach has been largely disappointing. Recombinant or synthetic sporozoite antigens protect some but not all of the volunteers^{3,4}, and the immunization of owl monkeys with a major blood-stage antigen of the malaria parasite *Plasmodium falciparum* was only partially effective⁵.

There are some reservations about sporozoite and blood-stage vaccines. The main arguments against a sporozoite vaccine are that only one sporozoite needs to escape to the liver to initiate an infection, and that in endemic areas people acquire high levels of anti-sporozoite antibodies but still become infected. An argument against merozoite or blood-stage vaccines is that although they might ameliorate clinical disease, they cannot prevent infection. The liver stages of the parasite have received relatively little attention because it has been widely believed that no immunity to these intracellular forms exists. The recent work by Schofield *et al.*¹ shows this assumption to be false, and also points to the central involvement of antibody-independent immune mechanisms in immunity to malaria.

Development of the liver stages of *P. berghei*, a malaria parasite of rodents, can be prevented *in vivo*⁶ and *in vitro*⁷ by administration of minute quantities of gamma-interferon (IFN- γ) — but if this is a transient phenomenon it is unlikely to be of any practical importance. Schofield *et al.*, also using *P. berghei*, treated rats immunized with attenuated sporozoites, a very effective method of vaccination, with a monoclonal antibody capable of neutralizing IFN- γ . This treatment not only permits infection of liver cells but also reverses established sterile immunity. The protective effect of IFN- γ is therefore not transient, but is an integral part of the immune response to malaria.

Several points concerning vaccination emerge from these experiments. Protective vaccination can be achieved by



Part of the immunoregulatory circuit. Parasite antigens are processed by macrophages (M) which act as antigen-presenting cells (APC). The first signal is interleukin-1 which activates two kinds of helper T-cells (T_H1 and T_H2). Further signals such as interleukin-2 (IL-2) activate lymphokine-activated killer cells (LAK) or cytotoxic T-cells while gamma-interferon (IFN- γ) activates further macrophages, and interleukins 4 and 5 (IL-4 and IL-5) activate B-cells. The net result is the production of cytotoxic T-cells, antibody, IFN- γ , tumour necrosis factor (TNF) and reactive oxygen intermediates (ROI), which separately or together can destroy malaria parasites. Interferons can also inhibit B-cell activity (broken line).

immunization with sporozoites or sporozoite surface antigens, and this protection probably involves antibodies. But similar vaccines are also effective in B-cell-depleted mice⁸, so antibody cannot be solely responsible for the induction of immunity. The depletion of CD8⁺ (suppressor/cytotoxic) T-cells also reverses immunity¹, which implies either that CD8⁺ cells might be cytotoxic, in which case they could kill malaria-infected hepatocytes by the same mechanism as they do in cattle infected with *Theileria parva*, another protozoan⁹; or that CD8⁺ T cells might be the source of the IFN- γ now known to inhibit the development of the liver stages.

It is unlikely that IFN- γ alone is an effector molecule in immunity to malaria. Elucidation of the immune response as a whole is beginning to revolve around our understanding of the various intercellular signals involved. Although the figure shows only some pathways, it is clear that IFN- γ could be involved either as a product of LAK cells¹⁰ or as a signal from TH1 cells¹¹ on macrophages triggering the production of reactive oxygen intermediates and/or tumour necrosis factor. A role for reactive oxygen intermediates in immunity to the erythrocyte stages of malaria has long been argued by Clark and his colleagues¹², and it would not really be surprising if immunity to the liver stages of the malaria parasite also involved a similar process, probably in combination with other mechanisms.

The possibility of developing a malaria vaccine has come a little closer as a result of the work of Schofield *et al.*, but it will not be straightforward. It is necessary to take into account the whole immune response, including its various regulatory hormones, and to manipulate these to drive the immune response along a desired pathway, perhaps by adding vectors containing interleukin-2¹³ or helper T-cell epitope¹⁴. It will not be sufficient to hope for an additive effect of humoral and cellular components of the immune system, because these may not act in a synergistic way. □

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Quantum chemistry

Helium can form stable bonds

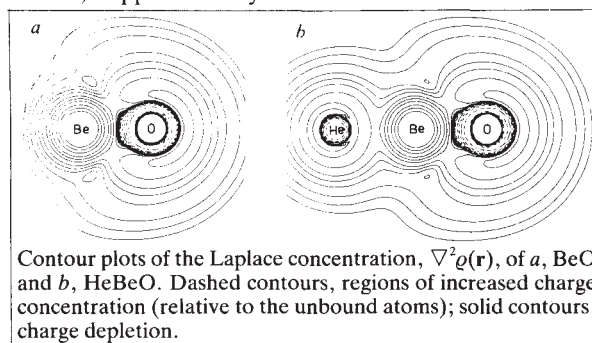
Neil Bartlett

OF all the noble gases, helium is the most noble. No neutral molecules are known that contain a helium atom, although helium does form ionic molecules and, in its excited states, transient 'excimer' molecules. In a recent paper, however, W. Koch *et al.* (*J. Am. chem. Soc.* **109**, 5912–5934; 1987) demonstrate persuasively with results from high-level computations that the linear molecule HeBeO is stable with respect to dissociation into helium and BeO, each species being in its ground state. The enthalpy for that process is endothermic by about 14 kJ mol⁻¹.

The first ionization potential of the helium atom (24.6 eV) is the highest known, approximately twice that of

essence of these long and detailed quantum calculations is to find the lowest energy distribution of electrons in the molecule. Also, the effect of zero-point energy was taken into account to evaluate enthalpies. Koch *et al.* show that helium forms strong bonds if the binding partner (acceptor) provides low-lying empty σ -orbitals. Electronegative atoms such as fluorine or oxygen are not suitable partners because they are electron rich. For similar reasons, HeOBe is not bound.

In its ground state (denoted $^1\Sigma^+$), BeO has a pair of core electrons on each atom ($1\sigma^2$, $2\sigma^2$), a triple bond ($3\sigma^2$, $1\pi^4$) and a non-bonding lone pair ($4\sigma^2$). The interaction with helium occurs through the



Contour plots of the Laplace concentration, $\nabla^2\rho(\mathbf{r})$, of *a*, BeO and *b*, HeBeO. Dashed contours, regions of increased charge concentration (relative to the unbound atoms); solid contours, charge depletion.

lowest unoccupied molecular orbital, 5σ . Koch *et al.* find that HeBeO is stable at all levels of theory. The figure compares the charge distribution in BeO and HeBeO. The Laplace concentration (which represents the second derivative of the charge density with respect to the coordinates) is used to give greater contrast to the areas of charge

xenon. The known (weak) bonding capability of xenon depends on electron donation (of which the ionization potential is a useful measure) to the atom or atoms to which it is bonded, and helium must, by comparison, be a much less effective electron donor. Indeed, it is the least likely species to form dative bonds. The high-level quantum-mechanical evaluations of Koch *et al.* show that BeO is an effective acceptor at the beryllium atom because that atom has effectively given its valence electrons to the oxygen atom in bonding to it. Consequently the BeO presents a hole of cylindrical (σ) symmetry at its beryllium end, and to an approaching helium atom that end appears to be approximately Be²⁺. All other electrons, which would have repulsive interactions with the helium electrons, are located on the oxygen atom and are consequently, in linear HeBeO, as far removed as possible from the He–Be bond.

In the Hartree–Fock molecular-orbital calculations, a rather large basis set (83 independent basis functions) was used to describe the wave function of the 14 electrons and fourth-order perturbation theory was used to account for the electron-correlation energy. Almost the same results were obtained at the multiconfiguration self-consistent-field level with a smaller basis set (35 basis functions). The

concentration and charge depletion in the molecules, relative to the neutral atoms. The picture of BeO shows that the beryllium has donated valence-shell electrons to oxygen, but that of the HeBeO reveals barely any further deformation of the electronic structure of the BeO and helium participants.

In contrast to their isoelectronic relative HeBeO, neither HeBN nor HeLiF is bound. This is because the Lewis acid (σ -acceptor) capability of BeO, BN and LiF is dependent on the effective charge at the σ -acceptor site (Be, B and Li, respectively). That effective charge is determined by both the nuclear charge of the σ -acceptor-site atom and the electronegativities of the atoms of the diatomic. Thus, the effective charge at lithium in LiF cannot be greater than +1 whereas, as the calculations show, the beryllium in BeO approximates to Be²⁺. But the boron in BN has a lower effective charge than the beryllium in BeO because of the higher electronegativity of boron relative to Be and the lower electronegativity of nitrogen relative to oxygen.

Preparation and identification of the HeBeO molecule presents an important challenge to the experimentalist. Although bound, the large increase in entropy associated with the dissociation to He and BeO means that thermodynamic stability

is only assured at low temperatures. Vibrational spectroscopic examination of gaseous reaction products quenched to liquid air or lower temperatures probably offers the best opportunity to observe the HeBeO species. It could be that HeBeO would be an effective source of ${}^1\Sigma^+ \text{BeO}$, if the lifetime of the triatomic, as seems likely, is greater than that of the diatomic. (Although strongly bound as a diatomic, BeO — like LiF and BN — rapidly polymerizes and cannot be isolated.) It could

be, however, that the most important aspect of this discovery is the superacceptor (σ -acid) property of ${}^1\Sigma^+ \text{BeO}$. This molecule will surely form Lewis acid-base adducts with many molecules other than helium. Even the poor bases (poor electron donors) CO and N_2 should form the stable linear tetra-atoms OCBeO and NNBeO . □

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Supernovae

Fewer and further between

Virginia Trimble

THE rate at which supernova explosions occur enters into our models of galactic evolution, interstellar gas dynamics and other phenomena. And the rates we have been using are too big by a factor of about three. So conclude¹ van den Bergh, McClure and Evans on the basis of systematic searches for supernovae in bright southern galaxies by Australian amateur Robert Evans. Five years' surveillance led to 15 discoveries, including six type II events (with hydrogen lines in their spectra) and nine type I (lacking hydrogen). But, according to the authors' interpretation of conventional rates² and of the search parameters, 54 supernovae should have turned up six type IIs and 48 type Is, a significant discrepancy.

What rates should we be using? The answer from van den Bergh *et al.*¹ applies to galaxies in the Shapley-Ames Catalog³, which contains a mix of types but is dominated by spirals rather like our Milky Way. For these, the total rate is $1.8 h^2$ per century per 10^{10} solar luminosities in a blue waveband ($10^{10} L_{\odot}^B$). The normalization by luminosity is clearly needed — bright galaxies will have more explosion-prone stars than will faint ones. The parameter $h = H_0/(100 \text{ km s}^{-1} \text{ Mpc}^{-1})$, where H_0 is your favourite value of Hubble's constant, expresses our uncertainty about distances to external galaxies and hence about their actual luminosities. The authors divide the $1.8 h^2$ supernovae among $1.1 h^2$ type II, $0.3 h^2$ type Ia (no hydrogen; found in old stellar populations), and $0.4 h^2$ type Ib (no hydrogen, but found in young populations) per century per $10^{10} L_{\odot}^B$.

For our own Galaxy, van den Bergh *et al.* adopt⁴ a luminosity of $2 \times 10^{10} L_{\odot}^B$ and so predict rates of 2.2, 0.6 and $0.8 h^2$ per century for the three types, or one supernova every 28 yr (because they belong to the $h \approx 1.0$ school of thought). Other constraints on the galactic rate are, at least, not inconsistent. Pulsars, generally supposed to come from type II supernovae, are born every 60 yr (ref. 4). Supernova remnants, which come from supernovae

surrounded by relatively dense gas, form every 60–80 yr (refs 5,6). Both these intervals probably have factor-of-two uncertainties. Finally, the seven supernovae seen in our sector of the Milky Way since 1006 AD imply an interval of about 30 yr between events over the whole Galaxy. If these numbers sound familiar, it is because van den Bergh *et al.* find almost no discrepancy with earlier results for the particular case of the Milky Way. Tammann expected² $4.2 h^2$ supernovae per century per $10^{10} L_{\odot}^B$ in a galaxy of our type. But he adopted $h = 0.5$ and a galactic luminosity of $4 \times 10^{10} L_{\odot}^B$. Thus, after also considering the historical events, he concluded that a galactic supernova occurs every 20–25 yr.

Predictably, the new lower rate is neither entirely unexpected nor entirely straightforward to interpret. In the past few years, several groups have concluded that supernovae as numerous as previously advertised would put more heat, kinetic energy and other disturbances into interstellar material than observations permit in E (elliptical) and S0 (lenticular) galaxies and other spirals^{8,9} as well as in the Milky Way¹⁰. Downward revisions by factors of two to three were required in all cases. The new rates seem to be just right.

They also fit well with a calibration based on deriving the birth rate of massive stars from the Lyman continuum photons which they produce and then asking how far down in mass one has to go among these stars to produce a given supernova rate¹¹. For the previous, high, rate, this was about five solar masses, down into the range that we think, on other grounds, actually makes white dwarfs rather than supernovae. Curiously, the new rate is not very different from that proposed by Zwicky¹² on the basis of his first three supernovae, found in 1,750 galaxy-years of searching. Because his search focused on members of nearby small groups, his average galaxy had $L = 10^9 h^{-2} L_{\odot}^B$ and his discovery rate corresponds to about $1.7 h^2$ supernovae per century per $10^{10} L_{\odot}^B$.

There remains one conceptual difficulty in interpreting the difference between the old and new rates. Type I events (at least type Ia events) are brighter than type IIs by about one magnitude or a factor of 2.5. Clearly this introduces some sort of differential effect into most searches, the fainter type II supernovae being discriminated against. Tammann made no attempt² to allow for this, assuming that all supernovae of both types in his fiducial galaxies and observation interval had been found. Thus his ratio of type I to type II supernovae in spiral galaxies is 1.2, the observed ratio in about 80 events actually recorded and typed. I would, therefore, have expected Evans to find a similar ratio, as indeed he did — $9/6 = 1.5$.

But the ratio predicted by van den Bergh *et al.* is $44/6$ or 7.3. What has happened is that they started with Tammann's rates, acted as if these had been corrected for a differential observational selection effect, and corrected back. The authors assumed Evans' search was complete to a particular level of apparent brightness (probably correct), calculated how many events should have risen above that level in the search period, and so concluded that the brighter type I supernovae should greatly outnumber the fainter type IIs on the discovery list. This was clearly to some extent the wrong thing to do. I do not know how precisely to describe the right algorithm, but it ought to predict roughly equal discovery rates for type I and type II supernovae in spiral galaxies.

Luckily this problem does not affect the reliability of the supernova rates finally adopted by van den Bergh *et al.*. These were derived by starting with the actual discovery list and allowing for differential selection effects. The result is that an observed type I to type II ratio of 1.5 is transformed into an occurrence ratio of 0.6. The main conclusion seems also to stand firm: Evans' predecessors were more thorough supernova searchers than we have given them credit for, and so the rates we have been using, derived from their work, were too large by factors of two to three. □

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Absentee ratio for boost-phase ASATs

SIR—For satellites at an altitude of 483 km, firing rockets with 6 km s^{-1} velocity, at boosters that burn for 300 s, the absentee ratio is 64, and it increases rapidly for shorter burn times.

Both supporters and opponents of the Strategic Defense Initiative (SDI) agree that an efficient boost-phase defence is critical. The main reasons are that the boosters are easily acquired targets and that destroying a booster destroys all its warheads and decoys. But if the satellites have altitudes of only a few hundred miles, they have a large absentee ratio, defined as the number of satellites that have to be deployed so that one satellite, on the average, is in range of the target at all times.

I have calculated the absentee ratio using numerical values from ref. 1, which gives the most favourable parameters for the defence that I have seen. They have the satellites at an altitude of 483 km (300 miles), firing rockets with 20-g thrust to attain a velocity of 6 km s^{-1} . Using these values, the value of the absentee ratio, a , is 64, for a booster burn time, t_0 , of 300 s; $a = 206$ for $t_0 = 180$ s (the boost phase of the MX missile).

The absentee ratio for satellites uniformly distributed in solid-angle (as seen from the centre of the Earth) is calculated below. If the satellite inclinations are optimized for a clustered launch at latitude 55° N , the absentee ratios are reduced by a concentration factor, y , which has values around two to three.

As seen from the centre of the Earth, a satellite at an altitude h firing rockets with a range $r = vt_0$, has in range all points within a solid angle $\Delta\Omega$, where

$$\Delta\Omega = 2\pi(1 - \cos \theta) \quad (1)$$

where θ is the angle between the vectors from the centre of the Earth to the satellite and to the target. The area accessible to the satellite is $\Delta A = R^2 \Delta\Omega$ (where R is the radius of the Earth), and the absentee ratio is $a = A/\Delta A = 2/(1 - \cos \theta)$, where $A = 4\pi R^2$ is the total area of the Earth.

If it is assumed that the intercept occurs above the atmosphere (about 100 km) but below the burn-out altitude (200 km for the MX), say at an altitude, h_0 , of 150 km, then the absentee ratio may be written as

$$a_1 = 4(R + h_0)(R + h)/[r_1^2 - (h - h_0)^2] \quad (2)$$

The average velocity during the first 30 s, while the rocket is accelerating to 6 km s^{-1} , is 3 km s^{-1} . To take that into account, subtract 15 s from the burn-time so that $r_1 = v(t_0 - 15 \text{ s}) = vt_1$.

I have used equation (2) to calculate a_1 in Table 1. The next row gives the concentration factor, y , by which the absentee ratio should be divided, if the satellites

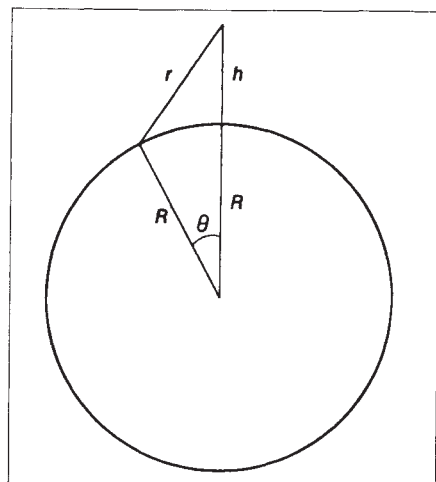


Fig. 1 Looking perpendicular to the plane containing the satellite (at a distance $(R + h)$ from the centre of the Earth) and the target (on the surface of the Earth) a distance r from the satellite.

are optimized for boosters launched from 55° N . This was calculated from a derivation by Garwin², which, for $a \gg 1$, gives $y = 2a^{1/4}/\pi[\sin(2I)]^{1/2}$, where I is the launch latitude.

Table 1 Absentee ratio and correction factors for various values of t_0 (the booster burn time in seconds)

t_0	a_1	y
120	625	3.28
180	206	2.49
240	104	2.17
300	64	2.08

The total number of satellites required for boost-phase defence is dictated by the target site with the most boosters and anti-satellite weapons (ASATs). If that site has 200 boosters and ASATs, and if, as in the Marshall Report, each satellite carries 10 KKV's (rockets), each of which makes a kill, then the busiest site requires 20 satellites. If we neglect the submarine-launched missiles and optimize for a launch from latitude 55° , the total number of satellites, N , is given by $N = 20 \times a/y = 615$ for $t_0 = 300$ s and $N = 1,655$ for $t_0 = 180$ s. Furthermore, for every additional 10 boosters or ASATs deployed at that site, we would have to deploy a/y additional satellites; 31 additional satellites for $t_0 = 300$ and 83 additional satellites for $t_0 = 180$. Finally, if we allow for missiles launched from widely different latitudes (for example, submarine-launched missiles), we lose the divisor 'y'.

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Isotopically depleted rainfall and El Niño

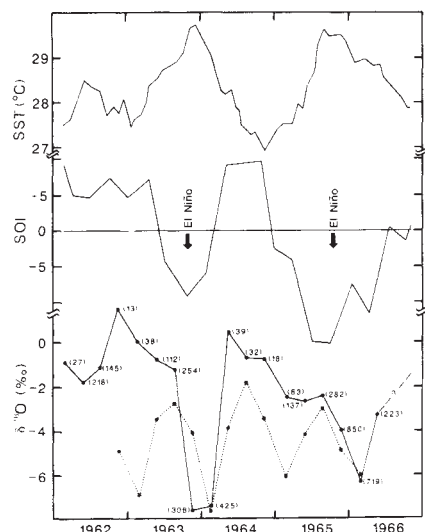
SIR—The El Niño/Southern Oscillation (ENSO) phenomenon is the single most prominent signal of interannual climatic variability, occurring every two to seven years when the upwelling of cold water off the coast of Peru is inhibited by weaker than normal trade winds^{1,2}. El Niño disturbances are accompanied by catastrophic rains in South America, and widespread drought in Australia and Africa³, the obvious human consequences of which have meant that a large effort has been mounted in recent years to understand, and ultimately predict, the phenomenon. Here I report historical data that suggest that El Niño events are associated with stable isotope depletion of the rainfalls in the equatorial Pacific.

Canton Island lies on the margin of the equatorial dry zone (2.77° S , 171° W , mean annual precipitation 748 mm; ref. 4), in the central west Pacific Ocean. Most of the island's total rainfall is associated with the heavy and widespread rains that fall in the central Pacific during El Niño events⁵. Therefore the island is sensitively placed to record variations in the stable-isotope composition of precipitation at such times.

Isotopic data is available for Canton Island^{4,6,7} for the period early 1962 to mid-1966, which coincidentally covers two El Niño events. Examination of the data reveals a marked depletion in the heavy isotopes of oxygen (and hydrogen) in rainfall associated with ENSO disturbances, with a change from 'normal' $\delta^{18}\text{O}$ values of -3 to $+2$ per mil, to depleted values of -6 to -8 per mil. As shown in the figure, allowing for a slight time lag, the isotopic data are strongly correlated with fluctuations in both the Southern Oscillation Index (the normalized sea-level pressure difference between Darwin and Tahiti²) and Canton Island sea surface temperature.

Similar depletions in the abundance of the heavy isotopes with increasing rainfall are well documented from tropical island stations (the so-called amount effect⁸) and are commonly associated with monsoon rainfall and movements of the inter-tropical convergence zone¹⁰. If the isotopic variations at Canton Island were simply due to the effect of the monsoons, then the variations would be an annual phenomenon, as in precipitation during the same period at other Pacific stations, such as Apia, Samoa (see figure).

Under normal conditions, monsoon rainfall does not enter the Canton Island area and the little rain that does fall is derived from the trade winds with an isotopic composition that is similar to that which falls year round at, for example,



Variation in the three-monthly amount-weighted mean oxygen-isotope composition of precipitation from IAEA/WMO stations at Canton Island (●—●) and Apia (●—●)^{4,6,7}. Canton Island sea-surface temperature (SST)¹² and Southern Oscillation Index (SOI)² for the period 1962 to 1966. El Niño events correspond to periods when the SOI is negative and SST is high. The $\delta^{18}\text{O}$ results are reported as per mil values relative to standard mean ocean water; numbers in parentheses represent the total precipitation in millimetres for each three-month period at Canton Island.

Rarotonga to the south east (approximately -2 to -3‰ for $\delta^{18}\text{O}$)^{4,6-8}. During an El Niño event, however, the South Pacific convergence zone, which usually oscillates on a seasonal basis between Fiji and Samoa (hence the strong seasonal monsoon effect on the isotopic composition of meteoric waters in that region) lies further east than usual and widespread rains result in the central Pacific^{3,5}. Precipitation at such times originates through convective uplift in the same way as isotopically depleted rains accompanying the monsoons, and therefore the central Pacific rains become similarly isotopically depleted. As a low-level easterly wind-stream develops along the equator during ENSO disturbances, additional vapour may also be added from the isotopically depleted rainfall province in the New Guinea-western Pacific area⁹.

The observation that isotopically depleted rainfall is related to El Niño disturbances rather than monsoon influences at certain tropical island stations is potentially of great use. Past isotopic variations of this kind may have been imprinted in the geological record in tree rings, parched lake sediments or lagoon corals. Information on the occurrence of El Niño disturbances in the past would provide important information about long-term fluctuations in periodicity that might aid in the prediction of future events.

Quinn¹⁰ considers that during glacial times the southern oscillation may have been arrested in its positive index phase

so that El Niño events would not have occurred. If this were the case, relatively drier conditions would have prevailed in the central Pacific and rainfall in the area would be derived only from trade wind precipitation. In such circumstances the isotopically depleted component of the rainfall would be absent. Such an absence might also be preserved in the geological record.

Dry conditions were necessary for the formation and preservation of the extensive Pleistocene guano-derived phosphate deposits of the tropical Pacific^{10,11} and studies of the oxygen-isotope composition of phosphates from the region¹¹ indicate equilibrium with waters isotopically similar to modern trade wind precipitation. Depleted compositions indicating equilibrium with monsoon precipitation, such as have been obtained from phosphates in the New Guinea region¹¹, are notably absent. This has been attributed to monsoon failure or increased evaporation, but the absence of El Niño disturbances might also be a contributing factor. Hence, additional isotopic work on phosphates and subaerially recrystallized carbonate material¹² from islands in the equatorial region of the Western Pacific could be of great use in elucidating climates and atmospheric conditions during glacial maxima.

A correlation between the isotopic composition of meteoric waters in the central Pacific Ocean and El Niño disturbances can hardly be considered irrefutable on the basis of the limited data available. However, given the importance of El Niño disturbances to so many aspects of global climate, the data do provide a strong case for the reactivation of central Pacific water sampling stations of the International Atomic Energy Agency/World Meteorological Organization network to investigate the relationship further, as well as for research to identify stable-isotope indicators of past El Niño events in the geological record.

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EDRF — a protective factor?

SIR—The recent demonstration by Palmer, Ferrige and Moncada¹ that vascular endothelium-derived relaxing factor (EDRF) is probably nitric oxide (NO) suggests that a physiological function of EDRF is to act as a chemical barrier to cytotoxic oxygen free radicals (O_2^-) from oxygenated blood.

The inactivation of EDRF by oxygen free radicals was recognized² before it was demonstrated that EDRF is nitric oxide. As the reaction of NO with O_2^- removes the oxygen free radical³, NO produced by endothelial cells may scavenge oxygen free radicals and protect vascular smooth muscle and other cells outside vascular endothelial lining. This hypothesis is supported by the observation that EDRF function (as assayed by vascular relaxation) is more pronounced in systemic arteries that normally contain oxygenated blood than in systemic veins with low oxygen tension⁴. The observation that EDRF inhibits platelet aggregation is also consistent with a protective role for EDRF⁵.

If this hypothesis is correct, the well-known reversal of acetylcholine vasodilation in the presence of endothelial cells to vasoconstriction in the absence of endothelial cells⁶ can be reinterpreted as follows: it is possible that acetylcholine normally produces vasodilation through its action on muscarinic receptors of vascular smooth muscle, but oxygen free radicals 'denature' the muscarinic receptor or a subsequent step in the vasodilator chain so that acetylcholine produces abnormal vasoconstriction. The contraction of vascular smooth muscle caused by acetylcholine in the absence of NO is, therefore, because the normal muscarinic dilator mechanism is not protected from oxygen free radicals by NO. This line of reasoning does not deny that EDRF causes vascular relaxation through a nonmuscarinic mechanism by acting as an 'endogenous nitrate' on soluble guanylate cyclase.

This hypothesis is supported by two points. First, by releasing NO from endothelial cells in vessels with cholinergic vasodilator innervation, acetylcholine would need to diffuse from the adventitial-medial junction, where the innervation ends, through the muscular media to the endothelium, where NO is generated. The NO would then diffuse back into the media to cause vasodilation. The acetylcholine released at the outside border of the media would have to diffuse past muscarinic receptors thought to mediate constriction before NO from the endothelium could overwhelm the constriction and cause dilation. Such a mechanism is not parsimonious. Second, 'sandwich' or cascade experiments using acetylcholine have not tested whether EDRF protects the smooth muscle muscarinic

receptor from oxygen free radicals⁷. Atropinization of an assay vessel will test only that EDRF has nonmuscarinic vasodilator properties. The central point of this hypothesis is that the muscarinic effect on vascular smooth muscle, when protected by EDRF, causes vasodilation rather than constriction.

Thus, it is possible that an important physiological action of NO produced in endothelial cells is to protect the native cholinergic vasodilator mechanism from oxygen free radicals, rather than acting as a second messenger from endothelial cells to vascular smooth muscle cells. This does not necessarily apply to all endothelium-dependent vasodilators, as some physiological blood-borne agents are very likely to act by causing EDRF release from endothelial cells⁸. But for acetylcholine, which is not physiologically present in blood, it may be useful to think of endothelial NO as an endothelium-derived protective factor.

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Sequence similarities in calcium-binding proteins

SIR—In their recent paper¹, David Swan and collaborators described the amino-acid sequence of a bacterial calcium-binding protein and pointed to its similarity to calmodulin. The regions of similarity correspond essentially to the positions of the potential calcium-binding domains in both proteins, but the fitting is otherwise not strong. The calcium-binding sites in the bacterial protein are not as evenly spaced along the polypeptide as in calmodulin. Domain II in the *Streptomyces erythraeus* protein is atypical and presumably does not bind calcium. Unlike calmodulin, the prokaryotic protein lacks overall sequence homology between its two halves.

At first glance these differences seem minor and allowed the authors to postulate a calmodulin-like regulatory function for the *S. erythraeus* protein. But its similarity with calmodulin loses much strength when the sequence is compared with another class of EF-hand-containing proteins, namely the invertebrate sarcoplasmic calcium-binding proteins (SCPs)².

These proteins are abundant in muscles of annelids, crustacea, molluscs and protochordates, and bind 2–3 calcium molecules with submicromolar dissociation constants. Like the vertebrate parvalbumins, they seem incapable of protein–protein interactions and probably serve only as intracellular calcium buffers. Sequence analysis³ shows a four-domain organization, with domains I and III always functional and domain II in crustacea and protochordates and domain IV in annelids also being functional. Aequorin, the bioluminescent calcium-binding protein from coelenterates, displays significant sequence similarity⁴ with the subfamily of SCPs, especially with annelid SCP (our unpublished data).

We observe that the *S. erythraeus* protein has more sequence resemblance with the subfamily of SCPs and aequorin. The spacing of the calcium-binding domains of *S. erythraeus* protein displays the same irregularity between domains I, II, III and IV, with linkers of about 20, 10 and 2 residues, respectively. The prokaryotic protein scores an overall sequence identity of 33.3 per cent with SCP of the annelid *Nereis*⁵ and of 28.6 per cent with aequorin. With the last two proteins, the *S. erythraeus* protein possesses an atypical domain II. In domain II, *S. erythraeus* protein is 8 residues longer than annelid SCP, but is of the same length as crustacean and protochordate SCPs and as aequorin. By fast-scan comparison⁶, the most similar Ca²⁺-binding proteins of the SWISS-PROT databank (EMBL data library) were selected and alignment scores⁷ between the *S. erythraeus* protein and the latter proteins were computed using the Dayhoff MDM-78 matrix. Highest scores (11.2) and 10.2) are obtained with *Nereis* and *Perinereis* SCPs; the next highest score (6.3) is with aequorin; lower, but still significant scores (4.7 to 3.1) are obtained with shrimp, scallop and *Amphioxus* SCPs. All other calcium-binding proteins present in the SWISS-PROT databank have scores below 3, indicating non-significant similarity.

We therefore believe that the prokaryotic protein belongs to the SCP subfamily and is designed rather as an intracellular calcium buffer than as an enzyme activator.

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Sex and recombination

SIR—In two recent papers, Burt and Bell^{1,2} attempted to test the Tangled Bank and Red Queen hypotheses by analysing the excess chiasma frequency (ECF) in relation to life-history parameters in mammals. The Tangled Bank hypothesis states that sex and recombination function to diversify the genetic profiles of an individual's offspring, thereby reducing sibling competition³. It is assumed that the greater the number of offspring produced the greater the levels of recombination required to reduce competition³. Burt and Bell found a weakly significant negative correlation between ECF and litter size (which they assumed equated with sibling competition). The Red Queen hypothesis states that sex and recombination result in progeny genetically distinct from their parents and grandparents and which are thus less susceptible to antagonistic advances made during the previous generation, particularly by their parasites^{3,5}. To test this hypothesis, Burt and Bell used the relationship between age at maturity (assumed to be synonymous with generation length) and ECF and found that there is a strong positive correlation even when body size is taken into account².

The predictions of the Tangled Bank hypothesis, and the suggested methods by which they could be distinguished from those of the Red Queen hypothesis, have been criticized by several authors^{6–8}. I also believe that the Tangled Bank and Red Queen hypotheses have not been adequately tested in Burt and Bell's analysis, for two reasons.

First, the Tangled Bank hypothesis has been inadequately tested by using the relationship between litter size and ECF. An important component of the potential competition between siblings is the degree of philopatry of the weaned offspring. I suggest the Tangled Bank hypothesis predicts that philopatric species should have greater ECFs than dispersing species.

In all mammalian species, offspring receive their initial nutritional requirements from their mother, via the milk. Although this may be a period of intense competition⁹, it is unlikely that the genetic diversity of the offspring will reduce this competition in a way that would enhance the inclusive fitness of the parents. After weaning, however, various different nutritional niches may be available for the offspring. The degree of dispersal from the natal area varies widely in mammalian species^{10, 11}. In some species, the young remain within their parents' home range or group, whereas in others the offspring disperse from their natal areas. Fecund species tend to have greater degrees of dispersal than less fecund ones¹². In large mammals with low fecundity¹³, offspring from different reproductive events may therefore occupy their parents' home

range and compete with their parents and each other. This could explain the unexpected negative correlation between litter size and ECF¹.

Second, it might be expected that competition between siblings should occur post-weaning and before dispersal at or around sexual maturity in dispersing species¹⁰. Age at maturity is highly correlated with body size¹³, but age at weaning is only weakly correlated with body size¹⁴. Therefore, the offspring of larger species will tend to remain together in the natal area even after they have been weaned. This will not be true for smaller species. The offspring of species with greater age at maturity are, therefore, likely to encounter greater sibling competition than those which mature earlier.

In conclusion, Burt and Bell's test of the Tangled Bank and Red Queen hypotheses using ECF frequencies was carried out using only partially valid hypotheses. I am not convinced that either theory can currently be discounted as accounting for the evolution of sex and recombination.

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SIR—Burt and Bell¹ used a correlation of excess chiasma frequency with age at maturity to support the Red Queen model over the Tangled Bank model as the selective factor maintaining genetic recombination. The theoretical basis of this has been discussed²⁻⁵. The test raises the practical questions of the suitability of the organisms used and the extent to which excess chiasma frequency is influenced by selection for variation among offspring.

Mammals are rather unrepresentative species and have good powers of dispersal, which reduces sib-competition. In some species such as chimpanzees *Pan troglodytes* and rhesus macaques *Macaca mulatta* (both used by Burt and Bell), relatives of one sex cooperate (negative competition) to defend territories. This makes it unlikely that they will show good evidence of sib-competition. Sedentary organisms with a large brood size would be better. Plants could provide the best examples, as beside sexual species, par-

tially asexual, outbreeding and selfing species could be used to compare excess chiasmata with age at maturity.

An increase in chiasmata does not necessarily increase recombination. For example, replacing a single interstitial chiasma with two near the telomeres because of interference reduces real recombination. Also, excess chiasma frequency is not entirely independent of chromosome number. It is uncommon for bivalents to have more than six chiasmata and excess chiasmata per bivalent seems to be a better parameter to measure. Dip-teran flies all have a low haploid number and low recombination index irrespective of a generation time of days or years, typically with no recombination in males⁶. Differences in length between individual bivalents may also have an effect if recombination levels which ensure a crossover in short bivalents tend to produce several crossovers in long bivalents.

Chiasma frequency variation and distribution between sexes and species of annual temperate grasshoppers with similar karyotypes suggests that recombination has little to do with age at maturity, one year from egg laying. Some species have many excess chiasmata, whereas in others males have a single, terminally localized chiasma in each bivalent arm so that there is no recombination in most of the genome. The females may show similar, opposite, or no localization⁷, and more or less excess chiasmata. Similar sex-specific differences are shown by *Triturus* (newts)⁸ and *Equus caballus* (horse)⁹. In lepidoptera, females (the heterogametic sex) lack recombination. Selection for or against recombination cannot explain differences between sexes as chromosomes from both sexes end up together in each offspring. Physiological differences must be the prime factor, if only by preventing changes in recombination in one sex. Meiosis is a long process and spermatogenesis may take weeks to complete. We have suggested⁷ that reduced recombination may streamline sperm production in some species, particularly in insects where females need only mate once for life, and the males are relatively small and fast maturing. A similar effect might apply to the mammals used by Burt and Bell¹, presumably mainly males, since quantitative data on female meiosis is rare. Those with rapid maturation probably have more mating opportunities per week than slow maturing types, and may produce more sperm per kg body weight per unit time.

A phenotypic character like excess chiasma number can occur under several different selection pressures, and a universal explanation is unlikely. The relative effect of the Red Queen and Tangled Bank models will depend upon the ecology of individual species. The response may be limited by other factors. The risk

in accepting Burt and Bell's conclusions is that the other factors affecting chiasma frequency will be ignored. As a general rule in evolution, if something is possible and selectively advantageous, some organism somewhere will be doing it. But two species may not do it for the same reasons.

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Protein kinase C and cyclic AMP pathways cross-talk

SIR—Levitzi¹ argues that in intact cells the most likely mechanism by which activation of protein kinase C by phorbol esters potentiates the synthesis of cyclic AMP is via G_i, the inhibitory G protein of adenylate cyclase. The phosphorylation of the α subunit of G_i by protein kinase C, which has been observed in platelet membranes², would remove tonic inhibition of cyclase activity. In support of his argument Levitzi states that the effect of phorbol esters is quantitatively similar to that of pertussis toxin³, which ADP-ribosylates and inactivates G_i. We would like to present an alternative hypothesis based on our recent studies with cultured cells⁴.

These findings suggest that there is a G protein that is sensitive to pertussis toxin and that may act, at least in some cells, as a mediator of 'cross-talk' between protein kinase C and adenylate cyclase activity. In its phosphorylated form the G protein would activate the catalytic subunit and its phosphorylation by protein kinase C would be prevented by pertussis toxin. Note that there are a number of recently identified pertussis toxin substrates to which no function has yet been ascribed⁵.

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The natives were friendly

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The National Geographic Society: 100 Years of Adventure and Discovery. By C.D.B. Bryan. Abrams, New York/Phaidon, Oxford: 1987. Pp.484. \$45, £35.

THE publication of this sumptuous, and thoroughly enjoyable, volume to celebrate 100 years of the National Geographic Society makes one realize how much its magazine has become a part of everyday life. Like many people, I first came across it at school, on wet afternoons when games were cancelled, though it was the occasional glimpse of a nipple, rather than all the photos of volcanoes or happy peasants, that excited our adolescent imaginations.

In later years disillusion set in. The photos were still magnificent and the facts in the text were true — but they were the wrong facts. The bright and innocent world of the *National Geographic* bore little relationship to the world described by the wireless and the newspapers. With its winsome style, and lack of analysis, the magazine was beautiful but trivial, a sort of *Reader's Digest* with pictures. It was not until much later, by which time I was a professional archaeologist, that I appreciated how much serious research was financed by the Society. Now, with the recent launching of its excellent technical journal, *National Geographic Research* (76 years after the idea was first proposed), and the current policy of good commercial popularization of science, the Society has intellectually come of age and has got its priorities right.

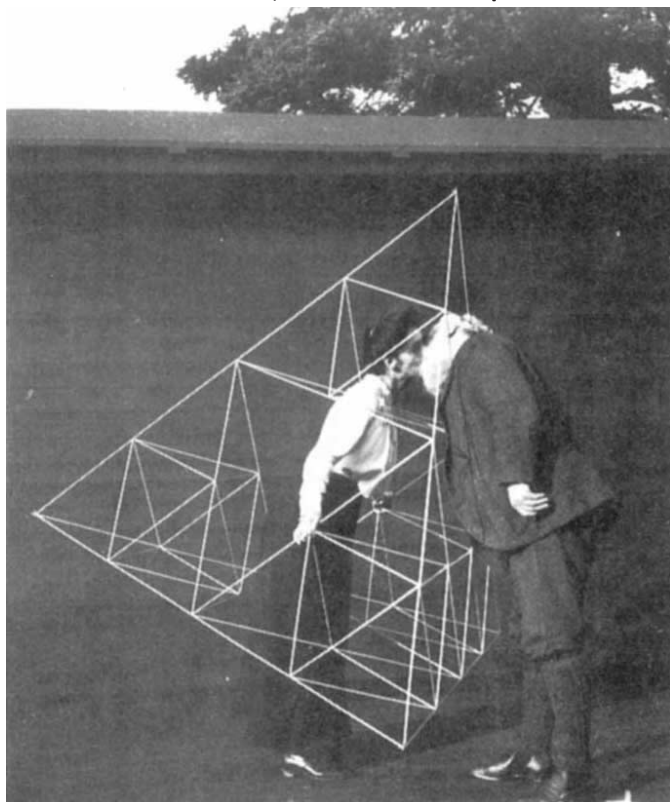
Bryan's historical survey confirms the general accuracy of these impressions. He interweaves, in alternating chapters, the history of the Society (and the behind-the-scenes debates over ideology and editorial policy), with thematic sections on, for example, the Society's contribution to polar exploration, mountaineering, archaeology, oceanography, aviation, space science and the development of colour photography. These sections provide a fascinating trip down memory lane, but they also show the importance of the Society's archives for future historians and social scientists.

The Society's photographers recorded everything, from major news events (such as the Bikini hydrogen bomb explosion of 1954, the *Columbia* space launch of 1981

or the eruption of Mount St Helens in 1980) to the everyday details of a world that is disappearing. Today it gives a genuine shock to find a black-and-white photo (from 1896) of a Zulu bridal couple captioned "These people are of a dark bronze hue, and have good athletic figures. They possess some excellent traits, but are horribly cruel when once they have

tions, refused advertisements for liquor, and also insisted that "Nothing of a partisan or controversial character is printed" and that "Only what is of a kindly nature is printed about any country or people, everything unpleasant or unkindly critical being avoided". This extreme conservatism might be construed as responsible journalism, and it was certainly good business (the Society today has subscribers in 170 different countries), but it sometimes had strange consequences. A 1949 article on the southern United States neither mentioned nor illustrated the black population, and the articles on Hitler's Germany and Stalin's Russia show the magazine at its anodyne worst. When, in 1977, realism was first introduced in features on Cuba, Harlem, Quebec and South Africa, the rumpus almost split the Society.

National Geographic Society



Age of innocence — "Dr and Mrs Bell demonstrate the lightness of both the inventor's tetrahedral structure and their hearts". Alexander Graham Bell became president of the National Geographic Society in 1898 on the death of its first president, his father-in-law Gardiner Greene Hubbard.

smelled blood". At this point one realizes that, after all, the text of *National Geographic* articles *does* matter, and that (despite its international pretensions) the magazine reflected, for most of its existence, the world view of small-town, white, Anglo-Saxon and Protestant America.

This theme is taken up in the historical sections of the book, and in the story of the Grosvenor dynasty that ran the Society almost as a family business. The first Grosvenor laid down the principles which determined both the strengths and the weaknesses of the magazine for more than half a century. He demanded strict factual accuracy and abundant illustra-

It is a measure of the book's quality that all these things are frankly discussed. Bryan was given the fullest cooperation by the Society, but he writes as an independent, albeit affectionate, outsider, and he has not produced a work of uncritical homage. Some people will read this book for the pleasure of its photographs and ragbag of facts. Others will appreciate that it also constitutes an important contribution to the history of science — not what scientists write about themselves, but science as perceived by the man in the street. The National Geographic Society has to be taken seriously. Its income is greater than the total science budget of many developing countries and, both as a patron of research and as an international opinion-former, it is an independent power in a world where high-level decisions on scientific policy are increasingly taken by politicians rather than by scientists

themselves. Worldwide, the Society has almost 11 million members, and many times that number read its magazine. That's a lot of voters. □

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● In the wake of Colin Renfrew's new book *Archaeology and Language* (reviewed in *Nature* of 28 January), Edinburgh University Press has just produced a second edition of Renfrew's *The Idea of Prehistory*, first issued in 1962. The revised edition contains two new chapters by Glyn Daniel which consider developments with the advent of 'New Archaeology' and radio-carbon dating. The book costs £19.50.

How much do you want to know?

Joseph Ford

Hamiltonian Dynamical Systems. Compiled and introduced by R. S. MacKay and J. D. Meiss. *Adam Hilger, Bristol/Taylor & Francis, Philadelphia: 1987. Pp. 784. Hbk £49.50, \$109; pbk £20, \$44.*

CREATORS of reprint anthologies appear to have no thought for the morrow, theirs or ours, for the product of their enthusiasm sweeps us all into a no-win/no-lose situation. Who can resist buying such a magnificent potpourri of reprint classics? Yet, who would wish to clutter his private library with such a mass of accompanying filler material? And thus our quandary grows: how many the classics, how numerous the fillers? Towards which side does the balance tip? Do the compilers know; do reviewers?

Consider the compilers. After diligently searching their memories and their reprint stacks for papers that deeply influenced their own research, they compose a table of contents, and in the process succumb to the temptation to add a few of their own articles to the list. Consultation with friends and colleagues fills in obvious gaps and extends horizons until severe dieting is required to bring the size of the book down to 800 pages. They then at last present us with a compilation which most assuredly includes many masterpieces; yet should not many of the included articles have been left out and vice versa? On judgemental issues of such delicacy, should or even can we trust anthologists not yet grey at the temples?

Consider the reviewers. The present reviewer, a physicist by trade, has favourably reviewed *Chaos*, an earlier reprint anthology published by World Scientific in 1984, and edited by the physicist, Hao Bai-Lin. Philip Holmes, a distinguished theoretical mechanician at Cornell, has written a scathing review of this same anthology. In particular, he deplores the physicist's myopia and the lack of appreciation for rigour that is reflected in Hao Bai-Lin's selection of reprints. Turning to *Hamiltonian Dynamical Systems*, one feels confident that Professor Holmes would rejoice in the extensive mathematical coverage of its subject area, whereas I am overwhelmed by its welter of formal articles which tell you much more than you wanted to know about mathematically rigorous developments. Given this consistent disagreement between tribal elders regarding the worth of anthologies, should or even can we trust such delicate judgemental issues to reviewers, no matter how grey they are at the temples?

If neither reviewers nor compilers suffice, how is a judgement to be made? Alas, in the face of such ambiguity, each buyer must reach his own decision; nonetheless, perhaps a few helpful hints can be provided.

At the outset, it must be emphasized that this anthology is strictly a reference work. Experts who read its table of contents, and find they know or wish to know most of the articles, should seriously consider the convenience of owning the book. Those less familiar with its contents should be wary, however, for many of the articles are highly mathematical and may prove quite opaque to all but the dedicated specialist. Moreover, there is little introductory or explanatory material to guide either the novice or the expert. Nonetheless, provision of the remarkably lucid, now classic review papers by Berry, by Moser, and by Arnol'd in the same volume with the seminal paper on Arnol'd diffusion, Chirikov's pioneering and almost readable paper on resonance overlap, John Greene's inventive but sometimes cryptic paper clarifying the notion of a transition to chaos, and Jack Wisdom and colleagues' charming paper on chaos in the Solar System is strong *prima facie* evidence that this book is worth the price.

And yet not everyone may wish to pay this price, because many do not belong to the audience to whom the volume is addressed. Recall that scientists tend to fall into two categories: those who accept without hesitation the notion that a simple closed curve divides the plane into an inside and an outside and those who do not. *Hamiltonian Dynamical Systems* is a volume created for those who do not! Once this determined bias towards rigour is recognized, the buyer is at liberty to accept or reject the volume.

There is, however, a much more serious

and yet much less obvious defect in this work, which lies in its presentation of a one-sided view of Hamiltonian dynamics. Its pages discuss quite remarkable progress, but always evolutionary progress in which today appears as little more than a magnified image of yesterday. Yet computational complexity theory, algorithmic complexity theory and cellular automata theory have long since established that chaos is defining limits on man's abilities no less revolutionary than those implied by *h* and *c*, and that Gödel, Turing, Church and Post are as relevant to Hamiltonian dynamics as they are to mathematical logic.

Although I cannot here even summarize all the issues involved, perhaps a simple question may provide at least the flavour of the thing. Existence-uniqueness theorems notwithstanding, how are we to assign a physical or a computational meaning to the notion of a chaotic orbit which not only has exponential sensitivity to slight inaccuracies in its initial state but which is also indistinguishable from a realization of some random process? A preliminary and partial 'practical' answer is provided by the Anosov-Bowen β -shadow lemma which, remarkably enough, does not appear to be discussed in this anthology; regardless, the logical issue remains unresolved because a chaotic (random) orbit contains more information than all of man's logical systems combined. How then are we to define the indefinable, compute the uncomputable?

MacKay and Meiss have in all truth compiled an excellent anthology for those who have developed a tolerance for being told both more and less than they wanted to know. □

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Firm foundations

David Oldroyd

From Mineralogy To Geology: The Foundations of a Science, 1650-1830. By Rachel Laudan. *University of Chicago Press: 1987. Pp. 278. \$27.50, £21.95.*

THIS volume is an invaluable addition to the small number of books in English on the early history of geology. Although, as Laudan's excellent bibliography shows, there is now an extensive secondary literature on the topic, there have been few attempts at synthesis of this material by authors who know the literature intimately and who have themselves contributed to it.

Geologists interested in the early history of their subject will probably be chiefly familiar with Adams's *Birth and*

Development of the Geological Sciences (1938) and Geikie's *Founders of Geology* (1905/1962). For Adams, study of early geology revealed the quaint ideas of our forebears. For Geikie, it demonstrated particularly the soundness of reasoning and method of early British (especially Scottish) geologists in comparison with their German contemporaries. Now Laudan skilfully displays the theoretical underpinnings of early geology, studiously avoiding any sense of disdain towards outmoded theories, or partisan leanings, and argues that the main framework for geological research in the early nineteenth century was set by the Wernerian School of Freiberg, rather than the Huttonians of Edinburgh. Geologists may also be interested, and perhaps surprised, to learn that Smith did not always practise what he preached. Although he undoubtedly enunciated and used the stratigraphical principle with which his

name is associated, in his mapping of England he sometimes relied primarily on lithological appearances and fell into error thereby.

Laudan lays particular emphasis on the methodological principles employed by early geologists, and seeks to display their several modes of reasoning. She distinguishes between 'causal' and 'historical' geology and, acknowledging that the latter came into prominence towards the end of the eighteenth century, she argues that a balance could be, and was, maintained between the two approaches. One may also distinguish between 'genetic' and 'historical' accounts of the Earth's past — that is between those that explain in terms of antecedent circumstances and laws of change, and those that explain by piecing together the contingent events of the past. Genetic accounts are subsets of causal accounts. Laudan deploys all three of these categories to advantage.

Laudan is also especially interested in methodological questions and distinguishes five approaches amongst the geologists that she examines: the methods of hypothesis, analogy, enumerative induction, eliminative induction and the (Newtonian) *vera causa* method.

The last of these was favoured in the nineteenth century, and Laudan contends that Lyell's celebrated uniformitarian approach displayed the method to fine advantage. A 'true cause' should be one that is known to be physically possible, and equal to its causal task. Inspection of present geological processes should enable both these criteria to be met, and hence Lyell could hold that his method fulfilled the methodological requirements of a 'Newtonian' science. It might seem that one scientist's *vera causa* is another's speculative hypothesis. Even so, Lyell's method did offer a possible route towards *verae causae* (though without certainty of success) and Laudan has surely construed his methodological goals correctly.

The book performs excellent service by displaying the institutional base of the German mining industry, and the Wernerian school which grew from it. There is, perhaps, room for more treatment of the institutional structures that began to develop in Britain and France in the early nineteenth century, and of the means adopted to certify geological knowledge. Personally, I should also have liked more information about the development of field techniques for mapping, drawing sections and so on. But, in general, one may say that a thorough task of synthesis has been performed. □

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A year in the life

Peter Coles

L'Année de la Science. By Roger Caratini. Seghers/Laffont, 6 Place St Sulpice, 75006 Paris: 1987. Pp.551. FF180.

ROGER Caratini and his publishers have set themselves an ambitious project — to publish an annual review, in French, of what's happening in contemporary science; or rather, what happened the previous year. By tackling questions at the leading edge of different disciplines, the intention is to present an up-to-date view of science as it really is, rather than as a static edifice of knowledge.

This 1986 debut amply confirms the author's determination not to popularize. Six 'hard' sciences — astronomy, physics, chemistry, biology, medicine and mathematics — are tackled with a detail that could dissuade readers without a professional or academic involvement with science. It is surprising, then, to learn that the target readership embraces high-school students, undergraduates, teachers and journalists, as well as researchers interested in peeking over the wall surrounding their own specialism to see what their neighbours are up to.

Usually, highly technical reviews are written by specialists for specialists and attract only marginal interest from outsiders. It is something of a shock, then, to discover that the author is not a science researcher, but an encyclopaedist, albeit one of the most experienced in France. This, as it turns out, is an advantage, because the yearbook has a didactic as well as an archival role.

Caratini's status as an outsider gives him a sympathy with his readers, who, he assumes, will expect memory-jogging and a few signposts to understand fields other than their own, but will appreciate the fascination of basic research. Having said this, alarm bells are bound to ring when a non-expert attempts a critical review of research in a specialist field.

Caratini is well aware of the scale of the task he has set himself and, to stifle the alarms, explains his approach. For the 1986 yearbook, he claims to have sifted over 30,000 research papers, finally choosing what to include on multiple criteria, such as frequency of citation. A scrutiny of the 1,200 or so references cited reveals a relatively high proportion of review articles and peer comments, and it is not clear to what extent these were used as the basis for selection and critical comment in the yearbook itself.

Even if the usefulness of *L'Année de la Science* as a critical review must remain doubtful, how well does it work as a reference source? Having lived with the book for a few weeks, I found it both interesting



Treasure trove — Californian acorn woodpeckers can store tens of thousands of acorns in a single tree for use during the winter. The picture, showing a typical granary, is taken from *Population Ecology of the Cooperatively Breeding Acorn Woodpecker* by W. D. Koenig and R. L. Mumme, which analyses the relationship of food-storing and cooperative breeding in this fascinating species. Published by Princeton University Press, the book costs Hbk \$55, £34.50; Pbk \$16.95, £10.60.

and useful. Earth sciences are not included; but if the 1986 index to *Nature* is taken as a reasonable guide to the main topics in different disciplines, it is otherwise nearly all here, roughly in proportion to coverage in the journal. AIDS (78 references in *Nature*) gets at least 15 pages, for example, while quasi-crystals (5 references) gets three.

The debates are clearly presented, with plenty of illustrations and useful 'foot-holds' in the margins — captions that give the gist of each paragraph. These turn out to be essential when the book is used for quick reference, enabling the reader to skip the introductory basics. At the back of the book is a 30-page glossary, which is a good, concise source of information in itself, and a complete list of Nobel prize-winners and Fields medalists.

The potential weakness of *L'Année de la Science* is that it tries to do too much — to be both a primer for the uninitiated and a reference work for the expert. The juxtaposition of different styles and levels of discourse is uncomfortable at times, and that it is not written in English will make it inaccessible to many potential readers. But the book should provide a welcome relief for scientists whose native tongue is French. □

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Wild variability

Dolph Schluter

Avian Genetics: A Population and Ecological Approach. Edited by F. Cooke and P.A. Buckley. *Academic*: 1987. Pp.488. £40, \$72.

RESEARCH on birds has had more influence on modern evolutionary theory than has study of most other taxa. Concepts as fundamental as clinal variation and the biological species were formulated with Aves in mind. Yet, although their systematics, behaviour and ecology have been extensively investigated, genetic processes in wild birds are still poorly understood.

This volume is the first to summarize what is known of avian population genetics. It contains a diverse collection of papers, written by a variety of researchers, but the material is reasonably well organized. Coherence is aided by the planning of the editors, whose overall intention was to characterize the different types of genetic variation found within species, and the demographic and ecological forces which shape it. Within the confines of this theme, we are treated to an excellent sample of approaches and questions in avian studies. Not all aspects of population genetics are included (for example species formation is hardly touched upon), but the book is a good beginning.

A particular strength is the attention paid to the many problems that remain. A wealth of questions is raised in virtually all the chapters, and many of these are highlighted by the editors in later summaries. The final chapter by Buckley is especially helpful in this regard, synthesizing advances and approaches to date, and pointing the way to the future. The result is a guide book for studies in avian genetics that will be useful to a fairly broad readership.

On the negative side, an excessive amount of the volume is devoted to basic methodology. This is especially true in the first section, which reviews the prevalence of many types of genetic variation in birds (Mendelian and quantitative traits, chromosomes, allozymes and DNA sequences). Several chapters would have benefited from briefer summaries of general technique, and greater discussion of the results of applications to work on birds. More frequent comparison with other taxa would also have been useful. A general conclusion to be drawn from this section is that birds indeed show genetic variation at all levels, but its significance is only dimly grasped and much remains to be catalogued.

The second part of the book deals with population processes moulding genetic

variation. The main processes are accorded separate chapters (inbreeding, gene flow, non-random mating, natural selection), and each is discussed in light of interesting examples. Here we begin to see the contribution that even straightforward assays of genetic variation in birds make to our understanding of evolutionary change. Some advantages of birds as subjects also become evident, such as the unique ability to mark and follow individuals. This feature allows Rockwell and Barrowclough to compare direct estimates of gene flow between populations with degree of population divergence. A second benefit of birds is their rapid and determinate growth, which simplifies measurement of morphology and estimation of its effect on survival and reproductive success. As a result, those working on birds are on the leading edge of investigations into natural selection in the field (here reviewed by Price and Boag).

The concluding section enlarges upon the evolutionary theme of the previous chapters. Included are reviews from an eclectic assortment of research projects

that have involved genetic studies. The players are familiar (great tit, house sparrow, snow goose and Arctic skua), though much recent work is described. The most interesting papers are those which manage to integrate genetic studies with other objectives in systematics, behaviour or population dynamics. A good example is that on the snow goose, by Cooke. Here, understanding of the elementary genetic basis of a plumage polymorphism has led to a detailed study of evolutionary processes on a continental scale.

The book is compelling reading because of the large amount of information reviewed, and the outline of many of the genetic and evolutionary problems which remain. In addition, one gains a better appreciation of both the benefits of genetic studies, and of birds as first-class subjects for research. □

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Twinkle, twinkle

Robert F. Garrison

The Classification of Stars. By Carlos Jaschek and Mercedes Jaschek. *Cambridge University Press*: 1987. Pp.413. £45, \$79.50.

CLASSIFICATION is a continuing activity for astronomers; we're not likely to run out of stars. Although it might be nice to have a detailed study of every star, it is far more practical to classify as many stars in as many ways as we can, and then to study the prototypes in detail. Our understanding of the nature of the Universe is improved by the development of better classification techniques. Of course, as fainter stars are surveyed in greater detail with better equipment, new categories present themselves, so the task of astronomical classification is never complete.

There is no comprehensive, book-length survey of the entire field of stellar classification, so the new volume by Carlos and Mercedes Jaschek fills a void. The authors have given us a wealth of information, which is accurate for the most part, and the list of references, though selective, is invaluable as a starting point for the study of any particular type of star. The level is that of the advanced undergraduate or beginning postgraduate student.

There are, however, a disturbing number of errors. One can forgive some mistakes of grammar, syntax, spelling and typography, but in this case the error level is about one per page, which is very much

higher than it should be. Most of these slips are just a nuisance and should have been caught by the copy editor, but some are more serious because they are subtle and change the scientific meaning of the passage concerned. The book in general is quite readable, but there is on occasion an irritating awkwardness in the choice of words. For example, "incertitude" is used instead of "uncertainty". While incertitude may well be correct, it is uncommon and (to me at least) appears stilted.

The idea of using tracings to illustrate spectra is a good one. Although photographic spectra might be easier to use in their original form, it is difficult to reproduce them without increasing the contrast and thus losing lines. However, it would have been better if the tracings had been from higher signal-to-noise digital spectra and if the spectra had been smoothed or broken into smaller segments. In some cases, the lines are hard to see, even for an expert.

The Jascheks have made a good attempt at organization of the field and its literature. Much of what I would like to see in such a book is there, though of course not always in the way I would like to see it. In a compilation volume, there is inevitably an author selection-effect, but that is the prerogative of the authors concerned and is one of the reasons for going to the trouble of writing a book. In any case, a student reading this volume would learn a great deal about stellar astronomy. I am using it, among others, for my course in stellar spectroscopy this term. □

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The thesis that won't go away

Beverly Halstead

The PhD degree is not an end in itself but a training for research work. The system of awarding doctorates for unpublished and unpublishable theses is overdue for reform.

IN THE career of a scientist, the degree of doctor of philosophy is one aspect of the rites of passage. But there is another role that this degree has taken on; it is becoming a measure for the calibration of the health of institutes of higher education, in that the speed of submission of theses is regarded as a barometer of the worth of the institution concerned. In Britain, the Economic and Social Research Council (ESRC) has taken the unprecedented step of blacklisting institutions where, if a certain number of candidates do not submit their PhD theses within a specified number of years, then no further PhD studentships are awarded to that particular institution.

The chairman of ESRC, Douglas Hague, noted¹ that half of ESRC's PhD students never obtain a degree: "The Council regards this as intolerable. If 50 per cent of first degree students in any field failed there would be an outcry. Why should PhDs be different?" Why indeed? It is a pity that Hague made no attempt to answer his own question.

It seems evident that the breakdown of the system is the direct consequence of how the PhD is viewed by students and academic staff. This has been well expressed as

... a question of failure to set reasonable expectations and to define exactly what the role of the PhD should be: at the moment, we are behaving as though it should be the life's work, rather than a period of apprenticeship which shows that the student is competent to become a professional researcher. We need to establish sensible criteria: a relatively short, self-contained piece of publishable work which demonstrates that the student has some grasp of how to use research methods and how to read journal articles, is an expectation that can be met. Eighty thousand words of struggling and endless effort to make a major contribution to Western thought is not the best way to demonstrate competence for the majority of young researchers².

Today, the PhD has become an ever-expanding, multi-volumed monster with a life of its own. But there is an unwillingness to return to first principles, to remind ourselves what exactly its purpose is. It is, I think, generally accepted that the purpose of a PhD is to train graduates to become competent research workers. It is assumed that everyone knows what this means but the assumption may not be justified. Before we can engage in worth-

while discussion, we have to decide what we mean by training in research and (first of all) what exactly it is that characterizes research. A good place to start is simply to observe what goes on. Immediately it becomes obvious that a PhD in biochemistry, for example, is different in kind from



one in the social sciences. Yet one aspect is the same across the board: a piece of research is not recognized as having been completed until it is communicated, and others know about it and have enough information to enable them to test its authenticity. The method by which this is achieved is by publication in refereed journals. A paper is prepared and submitted to a journal: the editor then sends the article out to an expert in the field for refereeing. Training in research cannot be said to have been accomplished if there has been no training in how to prepare the work for publication.

Looking at research scientists in action gives a clear indication of what their working life is like. The question is, does the preparation and submission of a thesis for the degree of PhD accomplish the avowed aim of training a young researcher for a scientific career?

The process leading to the award of a doctorate varies from country to country. In the United States it usually involves two years of taught courses, to provide a balanced background, followed by two years' preparation of a thesis. In Britain it involves three years' full-time research. In

the rest of Europe doctorates show a wider variation, and tend to be achieved later in life and to comprise a series of published works. In Scandinavia a doctorate is awarded for the public defence of research work published within a period of five years.

In Britain and North America, the production of a successful thesis involves supervision by an academic member of university staff, as well as technical assistance and the use of capital and consumable equipment. The return for many thousands of pounds or dollars of expenditure is a piece of paper that will ensure that the candidate will be able to secure a post at a higher salary than he or she could otherwise have anticipated, and a thesis on a library shelf. This is an expensive way to generate knowledge. Theoretically, theses are available for consultation, but the difficulty of obtaining access to them is such that in the main they are ignored and the scientific community does not benefit. A more serious objection, however, is that the candidate is allowed to indulge in a verbose expansiveness that no editor of a scientific journal would for a moment contemplate.

In 1971, in a letter published in *Nature*³, I voiced these points. No responsive chord was evoked by calling attention to the notion of cost-effectiveness. The Biosciences Graduate Committee of the University of Western Ontario, however, proposed changes in the regulations for the degree of PhD which required the publication of research findings before submission for the degree. This proposal was a direct result of the letter in *Nature*. At the University of California, Berkeley, and the University of Wisconsin reprints of unpublished articles alone may be submitted for the degree. At Stanford and Rochester, published papers, if retyped in thesis form, are acceptable. On occasion Case Western Reserve and the University of Manitoba have allowed the thesis to be substituted by papers but it is not a common practice. J.M. Prausnitz of Chemical Engineering at Berkeley had long insisted that his students wrote up their work in the form of journal papers. As he commented to me: "I have little doubt that the message of your letter will eventually be accepted by most of the academic scientific community. But it will not happen quickly".

In 1985 I again raised the issue in *Nature*, in much the same terms as before, but this time avoiding the emotive phrase of cost-effectiveness¹. This time the response was different, and a series of supportive letters followed^{5,6}. Mellanby⁷ reminded everyone that 20 years previously he had suggested that

... the thesis in its existing form be abolished and that candidates should submit published work or in the form required for a named journal. This would at least teach the candidate to read the instructions of the journal, something that many scientists of standing still do not do ... For what it is worth, my own PhD thesis consisted simply of a reprint of a 14-page paper published in the *Proceedings of the Royal Society*.

But by far the most encouraging response came from the Committee of Heads of University Geology Departments, which in November 1986 produced a discussion document (quoted in ref. 8) which included the following:

Students attempt to emulate the biggest or best of past theses. We must break into this spiral of rising aspirations and provide new aims, rules and guidelines for attainment of a PhD.

Some external examiners expect a rounded thesis of the kind produced about 20 years ago in very different circumstances. The PhD is a professional degree which should be considered as a report of a body of 'research progress', research that may be completed by the same or another scientist, after the completion of the PhD. Moreover students should be urged to consider publishing a proportion of this work after about a year so imparting the arts of precision and brevity and giving them encouragement in their quest.

New format: The PhD thesis should consist of several published papers, or manuscripts in publishable format with linking text, with the introduction and a literature review.

It was the views of the committee, quoting Mellanby with evident approval, that convinced me that there really was an emerging consensus on the matter. It seemed that this was the moment to put the new policy to the test. It is one thing to have all these views expressed — but quite another to have them translated into action.

At the time I had a part-time student who had been working on his project for six years, after having previously completed an MSc by research. He had already proved himself to be a competent research worker and his researches had been published in the leading international journal in his field. His PhD thesis was made up of five descriptive works, together with a synthesis — all the material had been published and had been subjected to peer review. His thesis was in my opinion an exemplary model of what a PhD should be.

Before the oral examination (which as supervisor I was prohibited from attending), I was permitted to explain to the examiners that, as the PhD was meant to

be a training in research, it was my contention that the research should be directed to preparing material for publication. I stressed that the large volume of work entailed in preparation for publication had been omitted and that the result was a thesis of commendable brevity. I drew attention to my letter in *Nature*⁴, and the responses it had elicited, and to the views of the Heads of University Geology Departments. I also pointed out that I and my supervisor had also submitted published papers alone for our respective PhDs,

"The question is, does the preparation and submission of a thesis for the degree of PhD accomplish the avowed aim of training a young researcher for a scientific career?"

which like my student's thesis consisted of descriptive works with a concluding synthesis.

The external examiners' report ran as follows:

... with some of the descriptive material he [the candidate] asserted, we believe honestly, that he was constrained by necessity to be brief in published work. There were several points where he was able to reinforce his descriptions and conclusions verbally in a way which we would have liked to have seen in the text. ... It was clear from the oral that he had an admirable knowledge of the palaeontology [of the vertebrate group he had studied] and of published work on their ecology and biogeography. Overall we were saddened to think that someone patently able enough to produce a satisfactory PhD thesis should have been inhibited from doing so by circumstances.

We applaud [the thesis's] lack of padding ... in length it is between one quarter and one third of that of successful PhD theses in vertebrate palaeontology which we have encountered. [But] we cannot convince ourselves that the work embodied represents a significantly higher ratio ... we cannot see it as much more than the equivalent of about a year's full-time study.

... in recommending that [the candidate] be awarded an MPhil. degree we both hope that he will exercise his right to decline it and then produce a thesis incorporating all his present thesis work plus some substantial body of [further] descriptive work.

The student accepted the MPhil. and has stressed that he has no complaint.

So, after six years of work, a steady stream of publications, clear evidence of being a professional researcher in his chosen discipline, he was offered yet another Master's degree, to my utter incredulity. The key point seems to be that the actual weight of text of the thesis was insufficient. It seemed to count for nothing that all of the material had been published, and no account was taken of the training aspect of actually preparing material for publication. This is a curious way of assessing work — exactly the same as an art critic judging a painting simply on

the criterion of the physical area of canvas actually covered by pigment.

In marked contrast to the slavish adherence to the vast, unpublished PhD thesis expected of students in Britain and North America, there has been a positive response from other countries. For example, a research student in Japan informs me that he must publish three papers in an appropriate journal and that

... these will become my doctoral thesis without presenting any special PhD thesis. ... This is the result of your comment on PhD in *Nature* in 1985. My professor read your column and blindly accepted it. A Japanese translation was published in the underground paleontological magazine *Plesion* and it was discussed and commented upon.

In Japanese universities there is no obligation for professors to educate students. The professor who has given neither guidance nor suggestions to his students says "OK, you must publish your paper in an international journal, then you can get the PhD. You must publish, if you do not, it is your failure." The professor can use your argument as a logical background for escaping promoting his students work: so what is his function? No guidance, no discussion, no promoting of students, he simply says "well a PhD should be given on published work."

This is going to extremes, but in the long run it will serve Japanese students well. Professors whose students do obtain the appropriate training will publish, and their success will be self-evident. Neglectful professors will have a negative record from their students, and injustices in the treatment of postgraduate students will be demonstrated for all to see. There are more checks and balances in awarding PhDs on published work than in the more enclosed system of preparing a massive manuscript that is not generally in the public arena.

It is perhaps most appropriate to end with the views of a research student, given in a letter to me:

During the course of my PhD I published a paper — it was far more exciting for me to do this than write a thesis. The former was a pleasure, the latter a chore. Do we really want to train scientists to write once in a lifetime theses rather than encouraging them to publish their work in the scientific literature? The people I know who have enjoyed their PhDs most are those who have successfully published papers during the three years of their PhD. Publication skills ought to be an integral part of any PhD training.

Amen!

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Polewards chromosome movement driven by microtubule depolymerization *in vitro*

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We constructed complexes between isolated chromosomes and microtubules made from purified tubulin to study the movement of chromosomes towards the 'minus' end of microtubules in vitro, a process analogous to the movement of chromosomes towards the pole of the spindle at anaphase of mitosis. Our results show that the energy for this movement is derived solely from microtubule depolymerization, and indicate that anaphase movement of chromosomes is both powered and regulated by microtubule depolymerization at the kinetochore.

THE mitotic spindle, a complex structure, mediates the correct segregation of sister chromatids during cell division^{1,2}. Chromosomes are attached to the spindle through the interaction of their kinetochores³ with the 'plus' ends of microtubules⁴ nucleated from spindle poles⁵. Within these structural constraints, polewards force is exerted on chromosomes to generate the steady movement of separated chromatids towards the poles during anaphase and the transient movement of paired chromatids towards the pole before anaphase⁶⁻⁸.

Chromosome movement is intimately linked to kinetochore microtubule dynamics. A chromosome can move towards a pole only when its kinetochore is connected to microtubules that extend from that pole⁹⁻¹¹. As chromosomes move on the spindle axis, their kinetochore microtubules change length, polymerizing during movement of chromosomes away from the poles and depolymerizing during movement towards the poles^{12,13}. Agents that cause microtubule depolymerization induce or accelerate the rate of polewards movement of chromosomes *in vivo*^{11,12,13}. Recent observations suggest that dynamic changes of kinetochore microtubules occur at the kinetochore—subunits are added to plus ends at the kinetochore during metaphase¹⁴ and are lost primarily at the kinetochore during anaphase^{14,15}.

Determining the role of microtubule depolymerization in polewards chromosome movement on the basis of *in vivo* experiments is complicated by our ignorance of the elements that may be acting in the chromosome or the spindle; many of these complications would be eliminated in a defined *in vitro* system

for polewards movement. Methods have recently been developed for isolating mitotic chromosomes whose kinetochores retain functional attributes including the ability to capture microtubules and to translocate along microtubules toward the plus end of the lattice, analogous to movement away from the poles inside the cell^{16,17}.

These observations encouraged us to develop an *in vitro* assay for movement of chromosomes toward the minus end of a microtubule, the direction chromosomes move during polewards movement. Here we describe the construction of a novel microtubule substrate and its use in detecting such movement. We examine the requirements for microtubule depolymerization during minus end-directed movement, the site at which depolymerization occurs and the nucleoside triphosphate requirement for movement.

Microtubule—chromosome complexes

To develop an assay for minus end-directed movement of chromosomes *in vitro*, we constructed a novel microtubule-nucleating structure, crosslinked biotinylated microtubule (CBMT) seeds (see Fig. 1 legend). These seeds are stable to depolymerization (half-life >20 min in <0.2 μ M tubulin), and are active in nucleating the formation of microtubules by the addition of free subunits to both ends (Fig. 1 legend). We made segmented microtubules in which unmodified tubulin was polymerized only from the plus ends of the CBMT seeds by elongating them with unmodified tubulin in the presence of tubulin modified with *N*-ethylmaleimide (NEM)¹⁸.

When we incubated chromosomes with segmented microtubules, we found three types of microtubules bound to the

Table 1 Statistical analysis of changes in microtubule lengths in segmented microtubule chromosome complexes

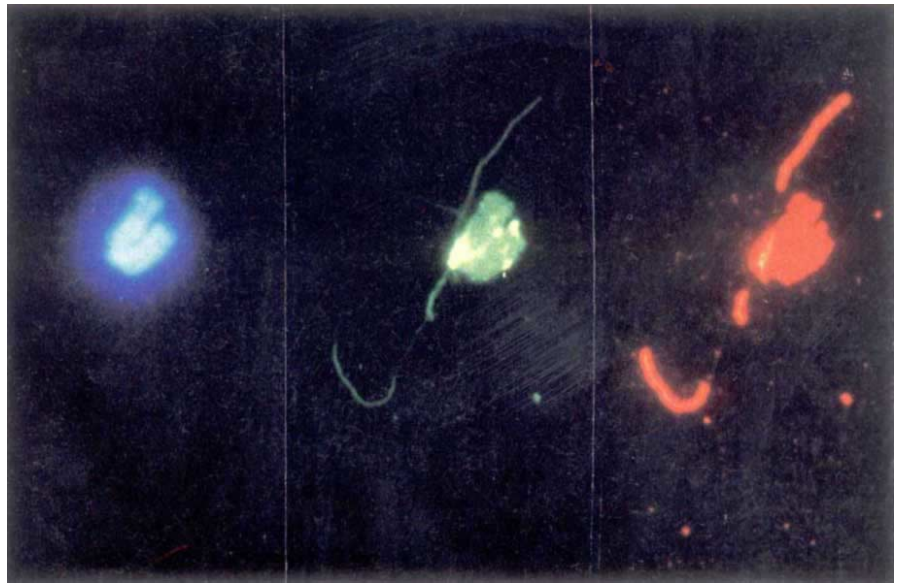
1	2	3	4	5	6	7	8	9	10
Concentration of tubulin in dilution buffer (μ M)	t_x min	FC t at t_x	FC < 6 at t_0	Sample number at t_x	FC < at t_x	Obs. no. C < 6 at t_x	Exp. no. C < 6 at t_x	χ^2	P
8	15	0.36	0.07	62	0.58	36	11	57	<0.01
5	6	0.29	0.04	18	0.67	12	3	26	<0.01
1	0.5	0.64	0.08	30	1.0	30	8	60	<0.01

To test the significance of the decrease in mean length of the microtubule between the kinetochore and seed (<6 μ m (FC < 6) for each tubulin concentration to determine whether the fraction of these complexes present at the end of the time course (FC < 6 at t_x , third column) increased relative to the fraction present before dilution (FC < 6 at t_0 , fourth column) by enrichment for pre-existing complexes with short segments through preferential loss of complexes with longer microtubule segments. The maximum number of C < 6 at t_x which could be expected to result from such enrichment (Exp. No. C < 6 at t_x , eighth column) was calculated using the equation (sample size at t_x) $\times$ (FC < 6 at t_0) / (FC t at t_x), where FC t represents the fraction of total complexes present at time x compared with the number present before dilution (Fig. 2). This equation assumes that all the complexes lost by t_x were ones which contained kinetochore to seed distances >6 μ m. To determine whether the number of C < 6 at t_x expected by this hypothesis could account for the number of C < 6 at t_x observed (seventh column), a χ^2 test was performed assuming one degree of freedom (ninth and tenth columns). In all cases the probability that these numbers are statistically the same is <0.001.

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Fig. 1 Segmented microtubules bound to the kinetochore of an isolated chromosome in which crosslinked, biotinylated seeds (CBMTs) elongated on their plus ends with unmodified tubulin have been captured at that end by the kinetochore of an isolated chromosome. Left, chromosome visualized by Hoechst staining of DNA; middle, the same field in the fluorescein channel in which total microtubules are seen; right, this field in the rhodamine channel in which only the CBMT portion of the microtubule is seen. Magnification 1,055 $\times$.

Methods. A 150-ml solution of PB (80 mM PIPES, 1 mM EGTA, 1 mM $MgCl_2$, pH 6.8) containing 30% glycerol, 4 mM $MgCl_2$, 1 mM GTP and 30 μ M phosphocellulose-purified tubulin³² was incubated for 20 min. This and all subsequent incubations were at 37 $^{\circ}C$. The solution was passed five times through a syringe with a 22-gauge blunt-ended needle. After 5 min, 3 μ l of this solution, containing many microtubule seeds, was added to a 100- μ l solution of PB containing 1 mM GTP, 0.05% β -mercaptoethanol, 30 μ M tubulin and 10 μ M biotin-labelled tubulin¹⁷. After 1 h the biotinylated microtubules had grown to an average length of 80–100 μ m. We added 10 μ l 15 mM ethylene glycol-bis-succinimidylsuccinate³³ (EGS) in dimethylsulphoxide (DMSO) to the reaction mix and crosslinking proceeded for 15 min. The reaction mix was very viscous before the addition of the EGS, so needed thorough mixing to ensure crosslinking of microtubules. This was done gently to avoid shearing the microtubules. The reaction was quenched by dilution into 1.0 ml solution of PB containing 50% sucrose, 10 mM glutamate and 0.5 mM β -mercaptoethanol, incubated for 1 h at 23 $^{\circ}C$, and then passed 4 times gently through a syringe with a 22-gauge blunt-ended needle to shear the microtubules to an average length of 7 μ m. The resulting CBMT seeds were stable for 2–3 months at 23 $^{\circ}C$ but not stable to freezing and thawing. Most (70–90%) of the CBMT seeds were capable of being elongated on both ends by the addition of free tubulin subunits. To construct microtubules in which tubulin subunits were added only to the plus end of the seeds, PC tubulin and tubulin-modified with NEM as described¹⁸ were thawed and added immediately to a final volume of 100 μ l of PB containing 24 μ M unmodified tubulin, 14 μ M NEM-modified tubulin, 1 mM GTP, 1 mg bovine serum albumin (BSA) per ml, 5 mM β -mercaptoethanol, 0.1 mM spermidine HCl, 0.05 mM spermine HCl. This solution was warmed to 37 $^{\circ}C$ over 1 min, and all subsequent incubations and fixation proceeded at 37 $^{\circ}C$. CBMT seeds (5 μ l, $\sim 2 \times 10^6$) were added and elongation of plus ends proceeded for 10 min. Isolated CHO mitotic chromosomes¹⁶ (10 μ l, $\sim 1 \times 10^5$) were added and incubated for 5 min to allow microtubule capture. An aliquot of the reaction mixture was fixed immediately while the rest was diluted (see below). Fixation of complexes using EGS and sedimentation onto coverslips was as described¹⁷, except that BSA (1 mg ml⁻¹) was used to coat coverslips as it gave lower background staining than poly-lysine. Immunofluorescent staining and microscopy was performed as described^{16,17}, except that we used triple labelling to see biotin. Coverslips were incubated sequentially with rabbit anti-biotin (Enzo), rhodamine-conjugated goat anti-rabbit IgG, rhodamine-conjugated rabbit anti-goat IgG, mouse monoclonal anti- β -tubulin, fluorescein-conjugated goat anti-mouse IgG and Hoechst 33258 at 10 μ g ml⁻¹.



kinetochore: segmented microtubules captured by their plus ends with the CBMT seeds distal to the kinetochore (Fig. 1); segmented microtubules captured by their minus ends with the CBMT seeds proximal to the kinetochore (not shown); and microtubules containing no seeds (Fig. 1) that were apparently nucleated from the kinetochore. Using our fixation procedures, we found very few microtubules non-specifically associated with chromosome arms. The complexes in which the plus end of segmented microtubules were bound to kinetochores are ideal for analysing minus end-directed movement of chromosomes, because the CBMT seed at the minus end provides a reference on the microtubule towards which chromosome movement can be monitored and also blocks depolymerization of the segmented microtubule from the minus end. Segmented microtubules bound by their minus end and nucleated microtubules are readily distinguishable and we ignored them when assaying minus end-directed movement.

Stability of complexes

Because polewards movement of chromosomes *in vivo* occurs concomitantly with microtubule depolymerization, we examined the fate of complexes between segmented microtubules and chromosomes under microtubule-depolymerizing conditions by diluting these complexes into solutions that differed only in the concentration of free tubulin. We determined the per cent of segmented microtubule-kinetochore complexes that remained as a function of time after dilution by dividing the average number of segmented microtubules per chromosome in a fixed sample at a given time after dilution by the average number in

a fixed sample before dilution. The concentration of free tubulin after dilution is an important parameter in the stability of segmented microtubule-chromosome complexes (Fig. 2). The rate of disappearance of the complexes was essentially zero at 20 μ M tubulin and increased as the tubulin concentration decreased from 8 to 1 μ M. Comparison of these stabilities with that of free microtubules is complicated, as under these conditions free microtubule ends exhibit dynamic instability¹⁹. However, most free microtubules grow at tubulin concentrations above 15 μ M and shrink below 8 μ M¹⁹. Therefore, segmented microtubule-chromosome complexes are stable under conditions that favour microtubule polymerization and are apparently unstable under depolymerizing conditions.

Length changes

We examined whether the length of the unmodified portion of the segmented microtubules changes as a function of time after dilution into tubulin by measuring the length of microtubules between the kinetochore and the CBMT seed on the same coverslips used to determine the stability of the complexes. Length measurements obtained from complexes that were diluted into 8 μ M tubulin are shown in Fig. 3A. We used similar data at each concentration of tubulin to calculate the mean length of the microtubule between the kinetochore and seed as a function of the tubulin concentration and the time after dilution (Fig. 3B). At ≤ 8 μ M tubulin, the mean length of the microtubule between the kinetochore and the CBMT seed becomes progressively shorter after dilution. At lower tubulin concentrations the mean length decreases at a more rapid rate.

Fig. 2 Stability of microtubule-chromosome complexes as a function of tubulin concentration. Segmented microtubules containing a CBMT seed at the minus end were captured by chromosomes. Aliquots of the complexes were diluted 20-fold into PB containing different concentrations of tubulin and 1 mM GTP, 1 mg BSA per ml, 5 mM β -mercaptoethanol, 0.1 mM spermidine HCl and 0.05 mM spermine HCl. Aliquots of complexes were fixed using EGS just before dilution and at various times afterwards, sedimented and stained. Chromosomes were selected for analysis that had readily observable primary constrictions (Hoechst staining) with microtubules (fluorescein channel) attached by their ends only to the kinetochore regions. Only then was the presence of CBMT seeds on those microtubules (rhodamine channel) analysed. The average number of microtubules with an unmodified portion of the segmented microtubule intervening between the kinetochore and the CBMT per chromosome was determined for each coverslip by examining ~200 chromosomes. The values obtained after dilution were normalized to the value obtained before dilution (time 0) to give the per cent segmented microtubules captured by their plus ends that remained bound after dilution. Microtubule-chromosome complexes were diluted to give final unmodified tubulin concentrations of: crosses, 20 μ M; open squares, 8 μ M; triangles, 5 μ M; closed squares, 1 μ M.

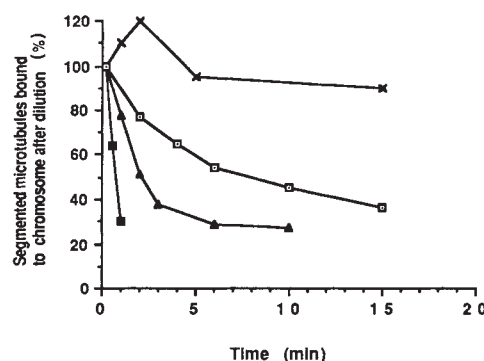
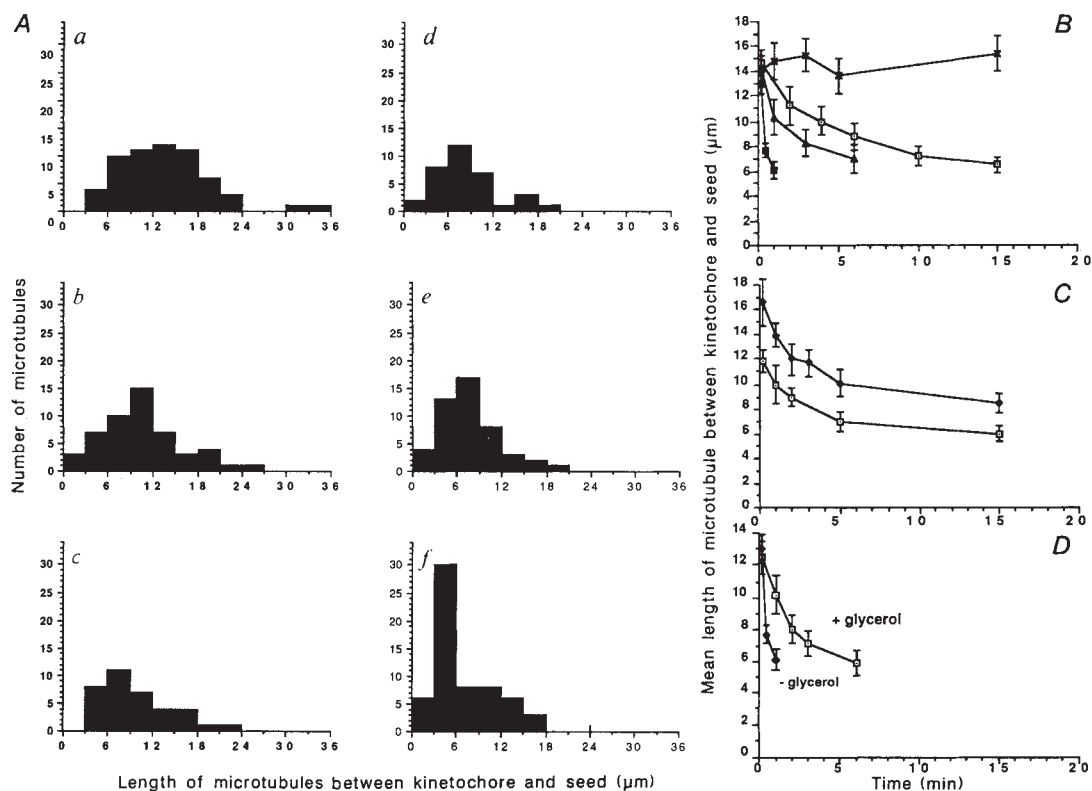


Fig. 3 A, Histograms of microtubule lengths between CBMT seed and the kinetochore measured at a, 0; b, 2; c, 4; d, 6; e, 10; f, 15 min after dilution into 8 μ M tubulin (see Fig. 2). The length of the microtubule between the CBMT seed and the kinetochore was determined for ~30 MT-chromosome complexes from each time-point by photographing images in the fluorescein and rhodamine channels. Images from the negatives were projected and superimposed by tracing and digitized³⁴. A histogram of those lengths for each time point is shown. Data for a was taken from the aliquot fixed just before dilution. **B**, Change in the mean microtubule length between CBMT seed and the kinetochore after dilution into different tubulin concentrations. Mean lengths determined from data as in A from the same assay illustrated in Fig. 2. Bars, s.e.m. complexes diluted into final unmodified tubulin concentrations of: crosses, 20 μ M; open squares, 8 μ M; triangles, 5 μ M; closed squares, 1 μ M. **C**, Mean length of the microtubule between the kinetochore and the seed before dilution varied by elongating CBMTs for 10 (open squares) or 15 min (diamonds) before addition of chromosomes. In both cases, complexes were diluted 20-fold to give 8 μ M final unmodified tubulin. **D**, complexes diluted 20-fold into buffer containing 10% glycerol to give final unlabelled tubulin concentration of 1 μ M (open squares). Dilution to the same tubulin concentration without glycerol is replotted from A for comparison (diamonds).



In dilution buffer containing decreasing concentrations of tubulin (20, 8, 5 and 1 μ M), the initial rate of shrinkage between the kinetochore and seed increases (0, 1, 2 and 12 μ m min^{-1} , respectively). These results indicate that the chromosomes remain attached to the plus end of the unmodified portion of the segmented microtubule as it depolymerizes. At high concentrations of tubulin (for example, 20 μ M), where most microtubules in solution elongate, we see no increase in length of the unmodified segment, unless ATP is added. This result is consistent with previous experiments showing that translocation of chromosomes towards the plus end requires added ATP¹⁷.

We considered two alternative explanations for the decrease in the mean length of the microtubules between the kinetochore and the CBMT seed. Because the starting population of lengths was heterogeneous (Fig. 3A) and complexes disappeared (Fig. 2), a reduction in the mean length could have been artificially produced by the enrichment for short complexes that existed before dilution through preferential loss of longer complexes after dilution, perhaps because of their greater shear sensitivity, for example. But a statistical analysis of the length histograms (Table 1) shows that the number of short complexes after dilution is too great to be accounted for by the number of complexes

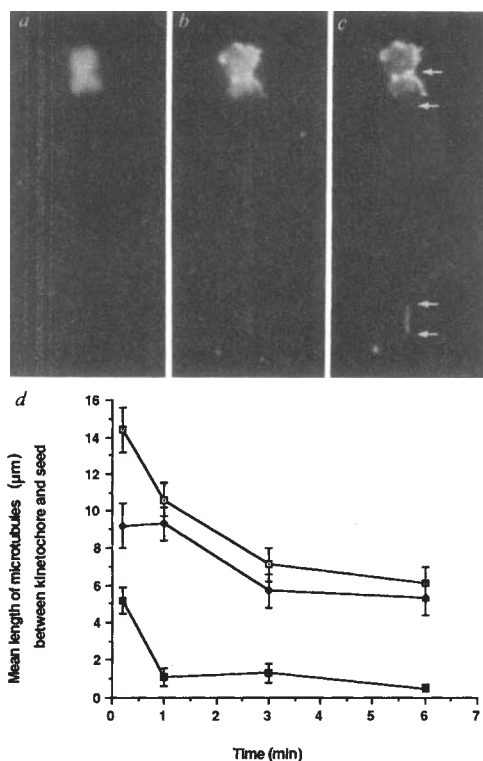


Fig. 4 Position of microtubule subunit loss during movement of chromosome from plus to minus end of the microtubule. A field containing a chromosome which has captured a tripartite microtubule by its plus end (fixed prior to dilution) is shown in *a* (Hoescht staining of chromosomal DNA), *b* (fluorescein staining of tubulin) and *c* (rhodamine staining of biotinylated tubulin). Microtubule minus end, lowest arrow. The CBMT is between this arrow and the next one up. The unmodified tubulin segment is between the two middle arrows, and the labile segment containing a low density of biotin between the top two arrows. Top arrow, kinetochore. Magnification 1,480 $\times$. *d*, Mean lengths of the unlabelled segment (closed diamonds), the labile biotin-containing segment (closed squares) and the total length of the microtubule between the kinetochore and CBMT (open squares) were determined for each time point. Error bars, s.e.m.

Methods. Tripartite microtubules containing the CBMT seed at the minus end, an unmodified portion in the middle and a portion containing a low density of biotin at the plus end were constructed as described in Fig. 1 except 0.5 μ l of biotinylated tubulin (10 mg ml⁻¹) was added 8 min after elongation of the seeds had begun with unmodified and NEM tubulin. This gave a ratio of biotinylated to unmodified subunits of 1:40 in the labile biotin-containing segment compared with 1:4 in the CBMT which could be distinguished by anti-biotin staining. These microtubules were captured by chromosomes and diluted 20-fold into buffer containing 10% glycerol to give 1 μ M assembly-competent tubulin. Glycerol was used for convenience to slow the depolymerization reaction. Aliquots of the complexes were fixed just before dilution and at various times after.

that existed before dilution, even assuming that none of these short complexes is lost during dilution. We also tested directly whether longer complexes are lost more rapidly by starting the dilution from two different mean initial lengths (Fig. 3C), and found similar shortening rates for the two populations. The second alternative explanation is that after dilution new short complexes are formed by the capture of free segmented microtubules that shorten with time. To assess directly the amount of new capture of microtubules after dilution, we added the same number of segmented microtubules to already diluted chromosomes. The number of complexes that formed when microtubules are added after dilution is <10% of the number formed

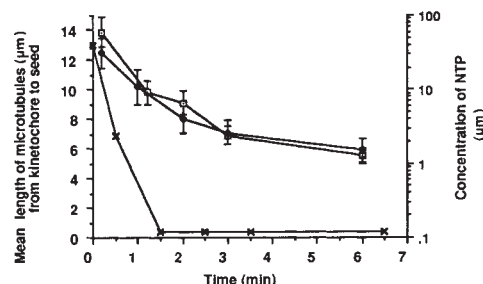


Fig. 5 Movement of chromosomes in the absence of nucleoside triphosphates. Experiment as described in Fig. 3D legend except that dilution buffer contained 10 μ M ADP, 20 U ml⁻¹ nucleoside 5'-diphosphate kinase (yeast type III, Sigma), and 10 U ml⁻¹ glycerokinase (*Candida utilis*, Sigma). Open squares, mean length of the unmodified portion of the microtubule from the kinetochore to the CBMT seeds as a function of time. Dilution in 10% glycerol and 1 mM GTP is replotted from Fig. 4c for comparison (diamonds). Concentration of NTP (GTP+ATP) before dilution and at various times after dilution is indicated by crosses and was determined by diluting 20- μ l aliquots in 200 μ l stop buffer (100 μ l equilibrated phenol and 100 μ l PB buffer) which immediately inactivated the NTP-depleting enzymes. The aqueous phase was removed and residual phenol in this phase extracted with ether. The aqueous phase was assayed for the presence of NTPs using the firefly lantern extract³⁵. GTP was used ~50% as efficiently as ATP by this system (data not shown). ADP provided a low-level background presumably because of low-level contaminating myokinase. The concentration of NTP at 1.5, 2.5, 3.5 and 6.5 min represents maximum values for the actual concentration in the dilution because these values are at the limit of the sensitivity of the assay.

in the standard assay. We conclude that the kinetochore remains attached to the unmodified portion of the segmented microtubule as it depolymerizes.

The rate at which the chromosome moves towards the microtubule minus end depends on the tendency of the microtubule to depolymerize. This conclusion predicts that agents that reduce the rate of depolymerization will reduce the rate of shortening. We examined the effect of glycerol, which decreases depolymerization rate²⁰. At 1 μ M tubulin in the presence of 10% glycerol, movement slows significantly from that observed in 1 μ M tubulin alone (Fig. 3D), consistent with this idea.

In all the assays in Fig. 3 the mean length of the microtubule between the chromosomes and the CBMT seed chromosomes did not decrease to zero but rather reached a plateau at ~40% of the initial length. The significance of this observation is unclear and it does not necessarily mean that microtubules started depolymerizing and then stopped. As the number of microtubules per chromosome decreased with time (Fig. 2), the mean length at later times could be biased by a small fraction (10–20%) of the initial population that failed to depolymerize.

Localization of disassembly

Although it seemed likely that the microtubule depolymerization occurs at the kinetochore, it was still possible that depolymerization was occurring along the entire length of the unmodified segment or at the juncture between the seed and the unmodified segment. To address this question, we constructed tripartite microtubules—a CBMT seed at the minus end, an unmodified segment of the microtubule in the middle, and a portion containing a low density of biotin subunits at the minus end (Fig. 4). By dividing the labile portion of the segmented microtubule in two, we could ask which part shrinks first, the unmodified portion near the seed or the low density portion near the kinetochore.

We diluted tripartite microtubule–kinetochore complexes into dilution buffer containing 10% glycerol to induce microtubule

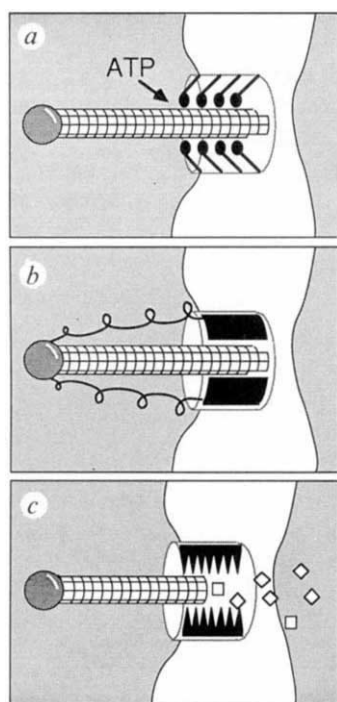


Fig. 6 Models for chromosome-to-pole movement. *a*, Translocating ATPase at the kinetochore that walks on the microtubule toward the minus end, where the pole (designated by a ball) is situated. This ATPase pulls the microtubule toward the kinetochore. *b*, The force exerted on the kinetochore pulling it towards the pole results from an independent structure, specifically an elastic element. The source of energy to stretch this elastic element initially is not specified. *c*, Force-producing movement of the chromosome to the pole is generated solely by microtubule depolymerization. The kinetochore remains attached to the microtubule as it depolymerizes.

depolymerization and determined the mean lengths of the unmodified portion and the portion containing the low density of biotin subunits as a function of time after dilution (Fig. 4d). The portion of the microtubule containing the low-density biotin label shrank completely before the unmodified portion began to shrink. The rate of change of the mean length of the total microtubule (Fig. 4d) is indistinguishable from that observed when segmented microtubule-kinetochore complexes are diluted into 10% glycerol (Fig. 3D), indicating that the presence of the biotin subunits at the plus end does not affect the process. These results support the hypothesis that depolymerization of the microtubule in segmented microtubule-kinetochore complexes occurs from the plus end of the microtubule at the kinetochore.

Role of nucleoside triphosphate

In all the preceding experiments, we added 1 mM GTP to the dilution buffer which, together with any ATP present as contaminant, could have provided the energy for chromosome movement. We therefore diluted segmented microtubule-kinetochore complexes into buffer that lacked GTP and contained a nucleoside triphosphate-depleting system of 10% glycerol, glycerol kinase, 5'-nucleoside diphosphate kinase and 10 μ M ADP. We determined the GTP+ATP concentration and the mean length of the microtubule between the kinetochore and the CBMT seed in parallel aliquots as a function of time after dilution (Fig. 5). The concentration of nucleoside triphosphate dropped to approximately 1 μ M in the first 30 s and to ≤ 0.1 μ M (the limit of detection of the assay) by 1.5 min after

dilution. However, the rate of change of the mean length of the microtubule between the kinetochore and the seed under these conditions is indistinguishable from the rate observed in the presence of GTP and no depleting system. The presence of 1 mM ATP in the capture reaction and in the dilution buffer also has no effect on the rate of change of the mean length (data not shown). Therefore, neither GTP nor ATP is apparently required to provide the energy for chromosome movement towards the minus end.

Discussion

Here we show that a chromosome can move towards the minus end of a microtubule *in vitro* under conditions that promote microtubule depolymerization. The following similarities were observed between the movement of chromosomes in the *in vitro* system and the movement of chromosomes during anaphase: in both cases kinetochores bind to the plus end of microtubules and move toward their minus ends³; movement rates range from 1 to 10 μ m min⁻¹^{1,2} and are decreased by glycerol, which slows depolymerization^{11,20}; microtubules depolymerize primarily near the kinetochore by loss of subunits from their plus ends^{14,15}; and finally, the total distance moved *in vitro* (7–10 μ m) is sufficient to account for polewards movement during anaphase A. These striking similarities between the properties of the *in vitro* system and those of the polewards movement of chromosomes *in vivo* strongly suggest that they occur by similar mechanisms.

Under conditions that promote microtubule polymerization, chromosomes seem to have a tight affinity for segmented microtubules (~85% of the complexes persist after 15 min). The affinity of chromosomes for segmented microtubules under these conditions is consistent with the half-life (15 min) of complexes between chromosomes and microtubules stabilized against depolymerization by taxol¹⁸. Therefore, microtubules bound to kinetochores and prevented from depolymerizing exhibit a stable association. This interaction may be responsible for the slow turnover of kinetochore microtubules compared with astral microtubules observed *in vivo*¹⁴.

The stability of microtubule connections to the kinetochore under depolymerizing conditions was investigated previously using microtubules nucleated from centrosomes and axonemes¹⁷. In that study, some complexes were detected 15 min after dilution, suggesting that the connection is very stable. But the frequency of such stable complexes (very low) was not determined, and it now seems likely that the complexes correspond to the small fraction of segmented microtubules that fail to depolymerize at late times in the present study. The significance of these more stable complexes is unclear; most plus ends at the kinetochore seem to depolymerize following dilution, while retaining the attachment. Thus, microtubules retain significant affinity for the kinetochore but this interaction does not greatly inhibit subunit loss. It would be interesting to assess the stability of the kinetochore-microtubule connection under depolymerizing conditions, where the chromosome and microtubule minus ends are tethered, to block shortening.

Many mechanisms have been proposed for driving polewards movements of chromosomes *in vivo*^{1,2,11,12}. The recent observations that kinetochore microtubule depolymerization occurs mainly at the kinetochore^{14,15} reduces the number of reasonable models to three basic categories^{21,22}: translocation of the chromosome along the surface lattice of kinetochore microtubules by an ATP-dependent molecular motor bound to the kinetochore (Fig. 6a); contraction of a parallel elastic element of matrix extending between the pole and the kinetochore²³ (Fig. 6b); or movement driven directly by microtubule depolymerization, with the kinetochore acting to transduce depolymerization into force²⁴ (Fig. 6c). Our present study shows that minus end-directed movement of chromosomes *in vitro* occurs in the absence of nucleoside triphosphate. In addition it is very unlikely to be driven by an elastic matrix; highly purified tubulin

was the only protein added to the chromosomes, and no matrix elements were observed by electron microscopy in an earlier study^{16,17}. We conclude that microtubule depolymerization is necessary and sufficient to drive minus end-directed movement of chromosomes *in vitro*, and suggest that the same is true *in vivo*. In support of this hypothesis, chromosome-to-pole movement in a lysed cell system²⁵ and calcium-dependent shrinkage of isolated spindles²⁶ both proceed in the absence of nucleoside triphosphates.

This hypothesis requires that the depolymerization of kinetochore microtubules releases sufficient free energy to account for the observed magnitude of polewards force during anaphase *in vivo*— 7×10^{-10} newtons per kinetochore, or 5×10^{-11} newtons per microtubule end²⁷. Assuming that microtubules shrink 0.6 nm for each subunit depolymerized, this force would require the depolymerization reaction to have a negative free energy of 7.2 kJ mol^{-1} . Each tubulin molecule hydrolyses one molecule of GTP when it polymerizes, and part of this energy is conserved in the structure and only released on depolymerization²⁴. As GTP hydrolysis has a negative free energy of $30\text{--}60 \text{ kJ mol}^{-1}$ *in vivo*²⁸, depolymerization could release enough energy to generate the observed force. To determine if the *in vitro* reaction is really a sufficient model for anaphase *in vivo*, it is necessary to determine how much force is generated *in vitro*. The force required to move a microtubule towards a chromosome at the rates observed in these experiments is $\sim 10^{-14}$ newtons (B. Nicklas, personal communication); this is less than the maximum *in vivo* force but still substantial. Because increasing the length of microtubules does not affect the rate of movement (Fig. 3c), movement in the *in vitro* system is not force-limited. Measuring the actual force generated requires further experiments.

How can the thermodynamic drive towards microtubule depolymerization be transduced by the kinetochore to provide the force for chromosome movement? Hill²⁹ proposed a model suggesting that depolymerization of the plus end of the microtubule at the kinetochore causes a biased diffusion of the chromosome towards the minus end (Fig. 7a). This model requires that the kinetochore has multiple binding sites for the microtubule and the energy required to move from one site to another is sufficiently small to allow the kinetochore to diffuse along the microtubule. Alternatively, a conformational change could occur at the plus end of the microtubule during depolymerization that exerts force directly on the kinetochore (Fig. 7b). In this model, fewer structural constraints are put on the kinetochore, which rides a conformational wave polewards. Curling up of protofilaments at the ends of depolymerizing microtubules³⁰ and depolymerization into curled fragments³¹, both seen by electron microscopy, are consistent with this idea. Further experimentation is required to determine the validity of these models. Whatever the detailed mechanism, we have demonstrated a novel motile mechanism—force production by

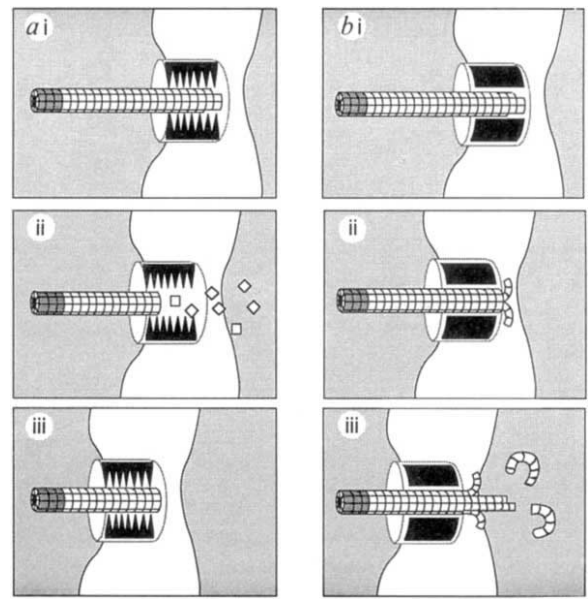


Fig. 7 Two models for chromosome movement driven by microtubule depolymerization. *a*, Biased-diffusion model based on the theoretical analyses of Hill²⁹. It is shown here in three discrete steps: (1) the microtubule is inserted into a sleeve which has multiple binding sites for the microtubule lattice; (2) depolymerization from the plus end causes no net movement of the microtubule relative to the kinetochore; (3) thermal energy causes a diffusion of the microtubule end so it penetrates deeper into the kinetochore, and the chromosome moves closer to the CBMT seed (stippled). The diffusion is biased in the direction shown because this increases the number of favourable interactions between the microtubule and the kinetochore sleeve. Continued loss of subunits and thermal fluctuations causes continuous movement of the kinetochore. *b*, Conformational-wave model. Depolymerization occurs by splaying of the protofilament structure and release of outwardly curved protofilament fragments. The splaying exerts a direct force against an annulus at the kinetochore leading to translocation of the microtubule towards the chromosome.

depolymerization of a cytoskeletal polymer—an idea proposed earlier by Shinya Inoue from purely *in vivo* experiments¹².

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Nickel, argon and cobalt in the infrared spectrum of SN1987A: the core becomes visible

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Infrared spectra of supernova 1987A taken in April and November 1987 from the Kuiper Airborne Observatory (KAO) show two distinctly different stages in the evolution of the expanding gas shell. In April both the optical and infrared spectrum originated from the hydrogen envelope, the still opaque remnant of the outer regions of the progenitor star's atmosphere. This region of the star was presumably of near solar chemical composition (although the elements heavier than helium are expected to have had an abundance from two to four times less than the Sun). Our April spectrum shows weak hydrogen lines rising above a 5,000-K photospheric continuum, in good agreement with this picture. By November, however, the spectrum of SN1987A had changed dramatically. Strong emission lines from heavy elements as well as many lines from highly excited levels of hydrogen dominated the spectrum with peak flux levels in the lines at or slightly above the level of the continuum in April. The intensity of these lines and the abundances of the heavy elements inferred from them demonstrate that the inner regions of the supernova are just now becoming visible at infrared wavelengths. These regions are expected to contain heavy elements produced by advanced nuclear burning stages in the progenitor star (Sk -69 202) and in the shock wave that ejected all material external to the iron core. They are, of course, very deficient in hydrogen.

These recent airborne observations of SN1987A in the 4–12.5 μm spectral range (Figs 1 and 2) show that, at present, the spectrum of the supernova looks very much like that of a dust-free, low ionization H II region dominated by emission lines from Ni I, Ni II, Ar II, Co II and H I. Conspicuously absent in the spectrum is the ground-state fine structure line from the Ni III ion at 7.348 μm . Additional lines from Ar III at 8.989 μm and Ni III at 11.002 μm are also absent in both our airborne data as well as spectra taken from the ground in early November (D. K. Aitken, personal communication), suggesting the absence of highly ionized atoms in the emitting region. Indeed, our November spectrum of SN1987A is completely consistent with theoretical expectations¹ of a low ionization state for material expanding from the inner core regions of the supernova. This low ionization is predicted by model calculations (ref. 1 and T. S. Axelrod, P. A. Pinto and S. E. Woosley, in preparation) as a consequence of the high density and strong cooling by the greatly enhanced abundance of metals. Such a low ionization state for the heavy elements raises serious questions about the tentative identification of strong emission lines at 10.52 and 18.28 μm in the supernova's spectrum with the S IV ion (D. K. Aitken *et al.* preprint; ref. 2.). We believe instead that the 10.5- μm line almost certainly arises from the Co II fine structure transition at 10.53 μm , in almost exact coincidence with the S IV line position. The line at 18.28 μm , identified with an excited state of S IV, can also be identified at least as well with an excited state transition in Ni II at 18.24 μm . The identification of these lines as singly ionized species is also consistent with the complete

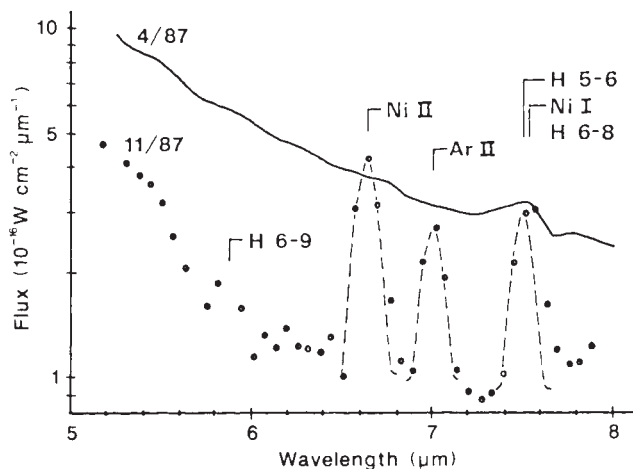


Fig. 1 Spectra of SN1987A from 5–11 μm , taken on 21 April and 12 November 1987, showing the development of line emission from Ni II, Ar II, plus a blend of H I and Ni I emission at 7.5 μm . Instrumental profiles are shown as dashed lines fitted to the data points of the November spectrum. A steep rise in flux shortward of 5.75 μm is due to emission from the 1-0 P branch of carbon monoxide. The formal standard deviation per data point is about $5 \times 10^{-18} \text{ W cm}^{-2} \mu\text{m}^{-1}$. Additional spectra covering the 3–13 μm range were obtained on 10, 12 and 14 November 1987.

absence of any higher-ionization lines, such as the [O III] $\lambda 5,007$ doublet, in the optical observations to date.

Preliminary analysis of our spectra indicate that the Ni II 6.6 μm and Ar II 6.98 μm lines are essentially unresolved (instrumental resolution is $\sim 5,000 \text{ km s}^{-1}$), while the H I–Ni I blend at 7.5 μm and the Co II line at 10.5 μm have significant widths. The width of the 7.5 μm blend is increased by the larger spread in velocity expected from the hydrogen lines which arise predominantly from the outer envelope of the material. Similarly the observed breadth of the Co II line is likely to be the result of a blend with an excited-state fine structure line of Ni II at 10.679 μm . Exact measures of the profiles of the observed spectral lines will require a much more detailed analysis of the data than is presented here. We find from our preliminary analysis of the data, using an instrumental profile derived from observations using the KAO and velocity resolution four times better Ni I $\approx$ H(5–6) at 7.5 μm , and Ni II* $\approx$ $\frac{1}{2}$ Co II at 10.5 μm . The velocity spread, Δv , for the H I lines in our data are consistent with $\Delta v = 2,500\text{--}3,000 \text{ km s}^{-1}$ while the lines arising from the heavy elements have a smaller spread $\Delta v < 2,500 \text{ km s}^{-1}$. Much better limits can and, we hope, will be placed by future observations using the KAO and velocity resolution four times better than in the flight reported. Models (ref. 3, for example) predict a lower bound on the hydrogen velocity near 2000 km s^{-1} and velocities for the iron group at $\sim 1,000 \text{ km s}^{-1}$.

Models also predict (ref. 3; T. S. Axelrod, P. A. Pinto and S. E. Woosley, in preparation) that the density of the core was $\sim 10^9 \text{ cm}^{-3}$ in November. At these densities, the infrared lines are at least marginally optically thick. One may thus obtain a rough estimate for the minimum velocity of the infrared line-emitting region from the fact that the monochromatic line flux in November was approximately equal to that of the continuum in April. This is of course a lower limit, as the lines are unresolved in these observations and are almost certainly narrower and hence brighter. If the temperature has remained roughly the same for the past six months (as the lack of colour evolution in the optical observations suggests), then the radius of the material emitting the observed lines at 260 days (10 November) must be the same as that producing the continuum in April (60 days). The velocity of the photosphere at 60 days is presumably less than that of the most slowly moving hydrogen, $2,100 \text{ km s}^{-1}$ (J. H. Elias and S. C. Gregory, in preparation), implying a radius of $< 10^{15} \text{ cm}$. For the nickel to have achieved a greater radius

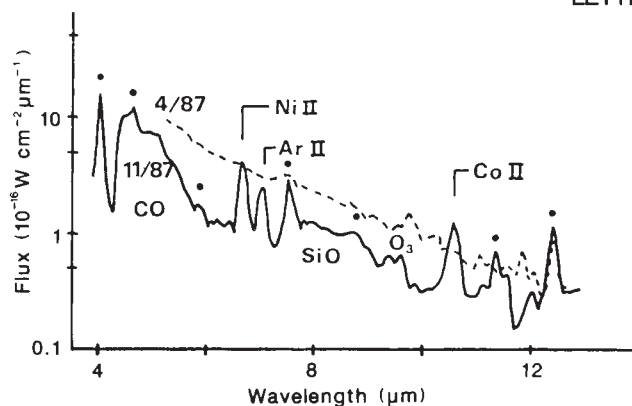


Fig. 2 Spectrum of SN1987A from 4 to 12.5 μm showing Ni II, Ar II and Co II emission lines on 12 November 1987 (solid curve). The spectrum on 21 April 1987 (dashed curve) is shown for comparison. H I lines are indicated by a filled circle. A region of poor cancellation of ozone absorption in the earth's atmosphere is marked by O₃ above the spectrum, and the positions of the 1–0 CO and 1–0 SiO emission bands are indicated by CO and SiO below the spectrum.

by November implies a velocity $\sim 1,000 \text{ km s}^{-1}$. In fact, the width of the H (5–6) line in the April data seems to be $\sim 4,000 \text{ km s}^{-1}$. If it were optically thick at that time, the inferred Ni II velocity would increase to $\sim 2,000 \text{ km s}^{-1}$. Models of supernovae without mixing of explosive burning products predict the velocity of the fastest moving cobalt to be well below 500 km s^{-1} . Thus, these observations provide further evidence of the core mixing invoked by several authors to explain the observed early, weak turn-on of X-ray emission near 180 days^{3–7}.

An estimate of the mass of Ni II which is responsible for the observed 6.6- μm line can be obtained from the observed luminosity in the line, $2.7 \times 10^{38} \text{ erg s}^{-1}$, and atomic data. At temperatures near 4,000 K and electron densities near 10^9 cm^{-3} , our measured flux implies a Ni II mass of $2 \times 10^{-3} M_{\odot}$. Because the density everywhere in the supernova is well above the critical density for these lines, and because $h\nu/kT \ll 1$, the flux in these infrared fine structure lines is independent of temperature and density to first order. It is determined only by the total number of ions in the ejecta. Comparable masses of cobalt and argon are also obtained by similar reasoning. We know from its light curve that the supernova has produced $0.07 M_{\odot}$ of ^{56}Fe (originally made as ^{56}Ni , most of which has by now decayed to ^{56}Fe), and we expect $\sim 4 \times 10^{-3} M_{\odot}$ of stable Ni (in all ionization stages) to be produced if Ni/Fe = 0.06 as it is in the Sun. Given the uncertainties in the atomic data for Co II, we can only say that the flux in the 10.53- μm line is consistent with the $8 \times 10^{-3} M_{\odot}$ of radioactive ^{56}Co expected to remain after 250 days. For a progenitor star with half-solar metallicity, the mass fraction of stable cobalt is $\sim 1.9 \times 10^{-6}$, leading to only $\sim 2 \times 10^{-5} M_{\odot}$ of stable cobalt in the star prior to explosion, an amount inconsistent with our observations. Thus, although we have strongly suspected its existence from the accurate tracking of the ^{56}Co lifetime by the optical light curve, these observations mark the first unambiguous detection and identification of spectral line emission, infrared or otherwise, by a radioactive element in a type II supernova (see ref. 8, and ref. 9 for an identification of radioactive Co III in the spectrum of SN1972e, a type I supernova). It is important to note that most of the cobalt responsible for the observed emission is radioactive, decaying with a half-life of 78 days; therefore, the decay of the 10.53- μm line with time, relative to that of the 6.63- μm line of (stable) nickel, and its eventual disappearance will be a critical test of its identification with ^{56}Co . We conclude by urging follow-up observations, especially of the Co II line at 10.53 μm , at higher spectral resolution to obtain valuable information on the velocity and velocity spread of the iron group elements synthesized in this explosion.

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The recombination wave and the hydrogen envelope mass of supernova 1987A

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The mass of the residual hydrogen envelope at the time of the explosion of SN1987A is an important parameter which is currently only weakly constrained by evolutionary models. The evolution of the photospheric radius in the first month is shown to be largely determined by the propagation of a hydrogen recombination wave into the ejecta. Provided that the fireball is expanding homologously and that the outer parts have a power-law density distribution, both the scale density and the power-law index can be determined from the observations. Here we show that the residual hydrogen mass was certainly larger than $2M_{\odot}$, and possibly as high as $12M_{\odot}$.

The explosion of the star Sk –69 202 as SN1987A was completely unexpected by observationalists and theoreticians alike. The star is an entirely unremarkable blue supergiant¹. Conventional wisdom had it that supernovae of type II occur either in red supergiants or perhaps in the Wolf–Rayet phase of evolution. The central problem for evolutionary models is therefore how the moment of core collapse can be contrived to occur in a blue supergiant star. There have already been many theoretical attempts made to address this question^{1–8}. These models have shown that the main-sequence mass must have been in the range 15–20 $M_{\odot}$ but that the end-point of evolution is remarkably sensitive to the assumptions and approximations made.

Theoretical estimates of the residual mass of the hydrogen envelope at the time of the explosion range from $\sim 0.3 M_{\odot}$ all the way up to $\sim 10M_{\odot}$. Several items of observational data tend to support the lower values. The strength of the nitrogen lines in the precursor⁹, and the appearance of narrow N V, N IV] and N III] lines in the ultraviolet some weeks after the explosion¹⁰ all suggest that CN-processed material was not only abundant in the surface layers, but indeed had been ejected in a previous red-giant phase. The early appearance of lines of s-process elements in the spectra¹¹, showing that He-burnt material is appearing at the photosphere, also tends to support lower values for the envelope mass of the pre-supernova star unless substantial post-supernova mixing has occurred. However, energy arguments would suggest high residual hydrogen mass, because in the low-mass scenario the hydrogen layers would be ejected at too high a velocity^{6,8,12}.

The compact nature of the precursor star ensured that adiabatic losses were considerable, and this was undoubtedly a factor contributing to the comparatively feeble optical display. The rapid expansion of the outer layers also allowed the photospheric temperature to fall rapidly. These factors allowed the hydrogen recombination wave to develop unusually quickly in SN1987A. Here the characteristics of this recombination wave are examined, and compared with observations. This is found to give useful restraints on the hydrogen envelope mass.

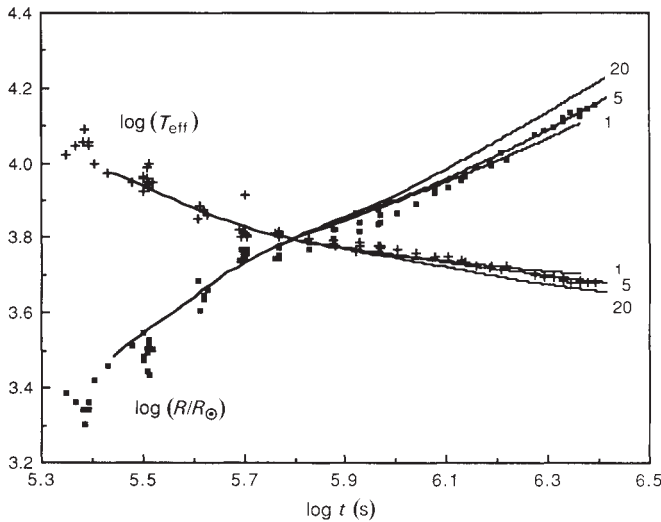


Fig. 1 The evolution of radius and temperature of SN1987A during the first thirty days. The theoretical curves are for initial parameters of $t_0 = 3$ days, $r_0 = 2 \times 10^{14}$ cm, $n_0 = 1 \times 10^{12}$ cm $^{-3}$ and opacities after recombination of 1, 5 and 20 times the electron scattering opacity of the residual electrons.

In the expansion phase, the photospheric radius is determined by the condition:

$$\int_r^\infty [3\kappa_\nu(\kappa_\nu + \kappa_{es})]^{1/2} \rho \, dr = 1 \quad (1)$$

where κ_ν is the absorption opacity and κ_{es} is the electron scattering opacity at frequency ν . We assume that at visual wavelengths, and for a fully ionized hydrogen plasma, the dominant opacity source is electron scattering. We also assume that the expansion is homologous, and that the density can be represented by a power-law of slope p :

$$\rho(r, t) = \rho(r_0, t_0) [r/r_0]^{-p} [t/t_0]^{(p-3)} \quad (2)$$

For constant opacity and ionization, equations (1) and (2) imply that the radius of the fireball $r \propto t^{(p-3)/(p-1)}$. However, when the temperature falls below a critical value, this assumption breaks down, and the photospheric radius is then dependent on the hydrogen recombination front and its propagation. The absolute luminosity of SN1987A during the first month¹³⁻¹⁷ can be accurately represented by the analytic approximation

$$L(t) \approx 3 \times 10^{41} \exp[-t/2.5 \text{ days}] + 1.5 \times 10^{41} \exp[-t/30 \text{ days}] \\ = 4\pi\sigma r^2 T_{\text{eff}}^4 \quad (3)$$

Below the photosphere, hydrogen is in, or very close to, collisional ionization equilibrium during the first month. The temperature, T , is given by the solution of the radiative transfer equation, and the ionization balance is determined by the recombination rate, β , and the ionization rate, γ , where

$$\beta = 3 \times 10^{-13} x n_e n_H [T/5,000 \text{ K}]^{-0.8} \\ \gamma = 1.1 \times 10^{-10} (1-x) n_e n_H T^{1/2} \exp[-95,000/T] \quad (4)$$

where x is the fractional ionization of hydrogen and n_e , n_H are the electron and hydrogen number densities respectively. This collisional ionization rate includes the enhancement caused by the effects of Ly- α trapping and by the ionization from excited levels (see, for example ref. 18). Above the photosphere, the temperature distribution is assumed isothermal and the time-dependent ionization is solved. The electron density falls until the metals with low ionization potential are the dominant source of supply of electrons. At abundances typical of the Large Magellanic Cloud¹⁹, this corresponds to a fraction 0.00014 by number. However, this may have been enhanced by an appreciable factor in the envelope of SN1987A. In any event, at these

very low electron densities, one can expect that the line opacity becomes the dominant opacity term.

The models show that, initially, the photosphere is locked to the steep outer boundary of the fireball. However, when the photospheric temperature falls to the point at which hydrogen recombination becomes important, a recombination front starts to move inward. From observation^{13,15,17,20}, this phase corresponds to 5–6 days after the explosion. The initial velocity of propagation of the recombination front is governed by the scale density at the outer part of the fireball, n_0 , because this determines the temperature gradient below the photosphere. Initially, the photosphere rides in with the recombination front. However, as the inward velocity of the recombination front increases, the opacity of the layers above the recombination wave becomes large, and the photosphere is left behind. The motion of the photosphere in mass coordinates is then determined only by the density power-law index, and therefore evolves with constant slope on the $[\log(r), \log(t)]$ curve. The observations show that this phase is reached by 16 days, and continues until about 25 days. The slope of the observed relationship in this phase gives $p = 10.6 \pm 1.5$. For the purpose of modelling we use $p = 11$, but also investigated $p = 9$ and 13.

The models are started at $t_0 = 3$ days; $r_0 = 2 \times 10^{14}$ cm, as defined by the observations²¹, and the scale density is determined by the motion of the photosphere at 6–12 days; 3×10^{11} cm $^{-3} \leq n_0 \leq 3 \times 10^{12}$ cm $^{-3}$. Figure 1 shows the excellent fit between the theory and observation so obtained for both the radius and photospheric temperature for three values of the post-recombination wave opacity, 1, 5 and 20 times the opacity of the residual electrons. The mass of gas which passes over the photosphere in the first month (assuming an He/H ratio of 0.09 by number) is, approximately:

$$[M/M_\odot] \approx 1.9 [n/10^{12} \text{ cm}^{-3}] 1.8^{(p-11)} \quad (5)$$

A more precise hydrogen mass estimate can be established by determining dynamically the lowest velocity of ejection of the hydrogen⁸, v_{min} . The infrared lines give the lowest value for this quantity, and they ought to be the most reliable because the optical depth in these lines is least. From spectra taken by P. McGregor (personal communication) at 104, 136 and 178 days, we find that the P β line gives minimum absorption velocities of 2,220, 2,520 and 2,320 km s $^{-1}$, respectively. From the observed photospheric (r, t) relationship, we find that this corresponds to the last of the hydrogen coming through the photosphere at $\log(t) = 6.85 \pm 0.05$ s. By integration of the density profile derived from the (r, t) relationship, this corresponds to a total hydrogen mass of $\sim 5(+7, -3) M_\odot$. This mass is consistent with only some of the current evolutionary models for the precursor star⁶⁻⁸.

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The ECORS deep reflection seismic survey across the Pyrenees

ECORS Pyrenees team*

The Pyrenees are an Alpine-age mountain belt located on the boundary between the Iberian and European plates. Using the same geological and geophysical data, Earth scientists studying the structure of the Pyrenees during the past ten years have not reached a consensus. Here we report the first results from a nearly continuous, 255-km-long reflection seismic survey across the Pyrenean chain. This first deep reflection profile of an entire orogenic belt will help to constrain possible models for the structure and formation of the Pyrenean chain.

The ECORS profile runs from the Ebro basin, near Balaguer, in Spain, to the Aquitanian basin, near Toulouse, in France (see Fig. 1). In the Spanish sector, the 150-km-long profile was obtained using 240 traces of geophones, 60 m apart, 20 kg of explosives situated at the centre of the spread being detonated every two traces in the first 70 km and every four traces in the last 80 km. Between the Spanish and French sectors, an 8-km-long, east-west profile was obtained entirely with the use of helicopters: 144 traces of 18 geophones, 60 m apart, were used here and a 20-kg charge was exploded every six traces. Further north, in the French sector, the spread included 240 traces of 18-geophone groups; the spacing was 40 m in the mountainous part and 80 m in the valleys. Four 13.5-tonne vibrators and two 7-tonne vibrators were used; in the first 24 km these were located in the centre of the spread. There was one vibration point (two vibrations) every 40 m. The remaining northern part of the segment (78 km) was obtained from 20-kg-charge shot-points arranged every six traces.

Classically, the structural zones of the Pyrenees have been defined¹, from north to south, as follows (see Fig. 1). The Aquitanian molassic foreland is overthrust from the south (along the Northern Frontal Thrust) by the North Pyrenean zone (NPZ), including Mesozoic flysch deposits and Hercynian basement cores (the North Pyrenean masifs). This zone is bounded to the south by the vertically faulted North Pyrenean Fault zone (NPFZ) running east-west along the belt, locally with highly strained Mesozoic metamorphic rocks^{2,3}. The Axial Zone (the highest part of the chain) is made of Hercynian material reworked during the Alpine orogeny by a southward-directed thrust system. In the South Pyrenean zone, Mesozoic and Cenozoic rocks, belonging to a large detached unit, are overthrust to the south over the Ebro molassic basin in the vicinity of the Sierras Marginales⁴⁻⁶.

The general fan-like geometry of the chain, with northward thrusting in the north and southward thrusting in the south, is one of the main surface characteristics of the Pyrenees. In this context, the very rapid variation of strain and metamorphic conditions observed along a north-south section is unusual: the highly strained rocks are restricted to a very narrow vertical area, the width of which is between 1 and 5 km (ref. 2). These rocks have been regionally metamorphosed at low pressure and high temperature. In some places, slices of mantle rocks (Iher-

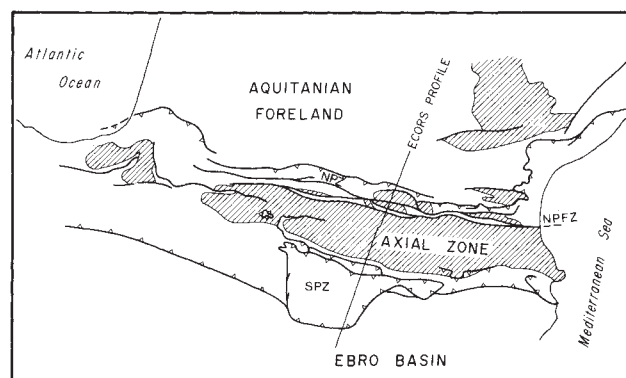


Fig. 1 Main structural units of the Pyrenees and location of the ECORS profile. NPZ, North Pyrenean zone; NPFZ, North Pyrenean Fault zone; SPZ, South Pyrenean zone. Diagonal ornament marks the areas where Hercynian basement outcrops. The French section of the profile 105 km long, was surveyed by the Institut Français du Pétrole during autumn 1985. The Spanish section, 150 km long, was obtained during autumn 1986 by Hispanoil. This profile was chosen from four possible locations in order to obtain the most representative section of the belt, where the various structural units are expressed and well defined at the surface.

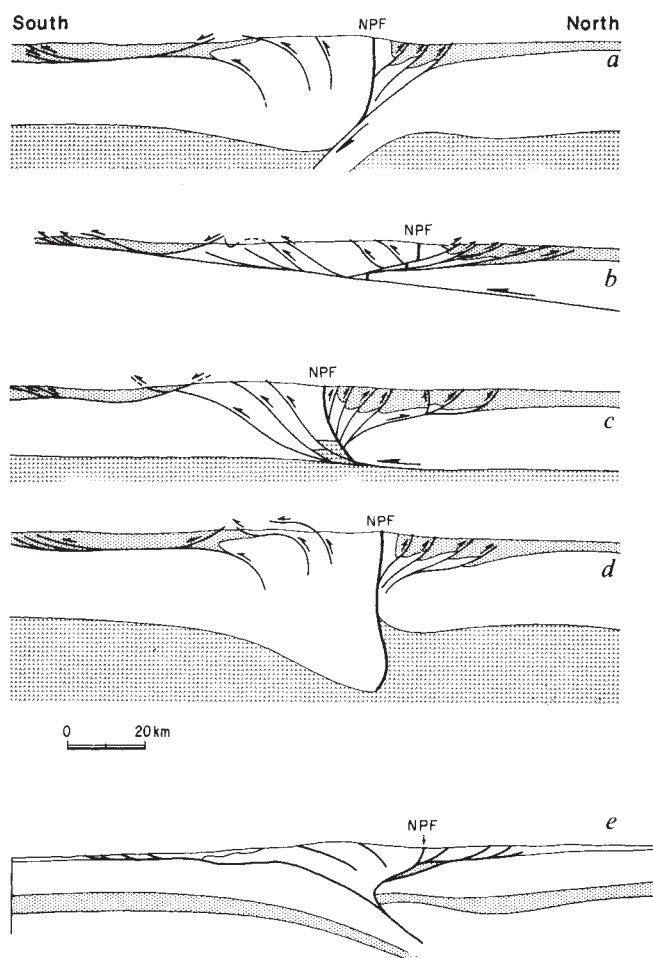


Fig. 2 Cross-sections presenting different hypotheses for the present overall geometry of the Pyrenean fold belt, according to refs 12 (a), 13 (b), 14 (c) and 1, 3 and 15 (d). NPF, North Pyrenean Fault. Section e presents a possible solution consistent with the ECORS data: the NPF is considered to be a Lower Cretaceous transform fault active during the earlier stages of the evolution of the chain, and deformed later, during the orogeny.

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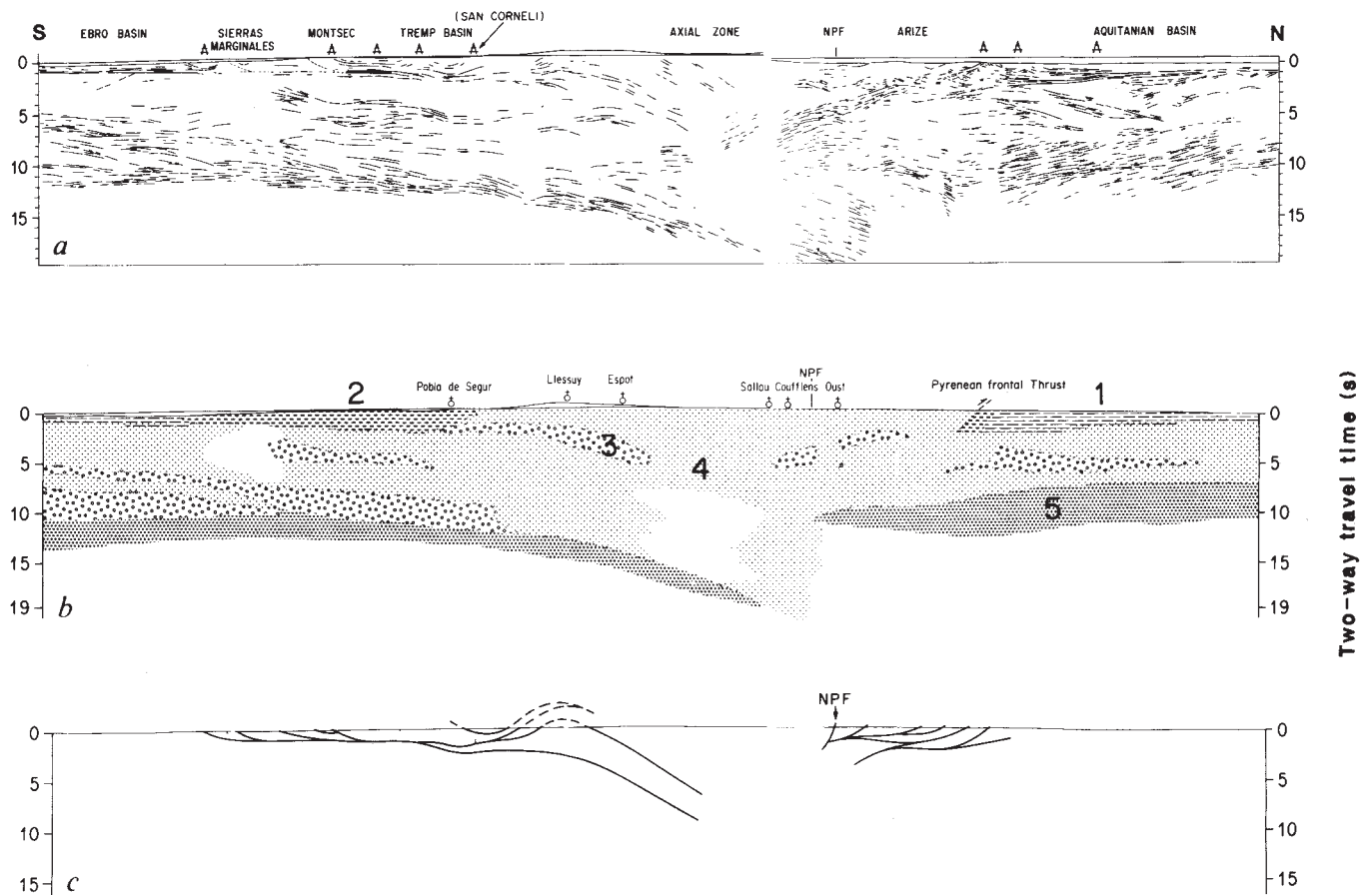


Fig. 3 Data from the ECORS profile. *a*, Line drawing of the entire profile. *b*, Main seismic facies along the profile: 1, undisturbed reflectors within the sedimentary basins; 2, same reflectors tectonically disturbed (see Fig. 4*a*); 3, oblique reflectors of the middle crust (see Fig. 4*b*); 4, domain of poorly defined reflectors; 5, layered deep crust (see Fig. 4*c*). *c*, Geometry of the major external thrusts from geological and seismic data. The central part of the fold belt is not represented.

zolites) occur, following the trace of the vertical North Pyrenean Fault zone, which appears to be the main linear feature of the belt.

Before the ECORS programme, it was already known that the Moho discontinuity is offset by >10 km below the surface trace of the North Pyrenean Fault (NPF)^{7,8}. The NPF has been interpreted as a pre-organic transform fault along which a sinistral drift of the Iberian plate occurred during Albian times^{9,10}. This displacement is thought to have opened the Bay of Biscay, involved crustal thinning in the North Pyrenean zone and caused the development of Lower Cretaceous pull-apart basins¹¹. The end of this episode of drift coincided with the deformation of the fold belt between Upper Cretaceous and Upper Eocene times.

Despite good agreement concerning the general structure near the surface, the geometry of the main discontinuities of the belt and the deep structure of the Pyrenees are still highly controversial: one can distinguish four main recent models (Fig. 2*a-d*). The first (Fig. 2*a*) posits that, after the drifting of the Iberian plate, Pyrenean deformation resulted from minor southward-directed subduction¹². The second is a thin-skinned tectonic model (Fig. 2*b*), incorporating a 60-km offset of the NPF at depth, by a major sole thrust dipping north at 6° (ref. 13).

Using a thick-skinned tectonic model (Fig. 2*c*), others consider that the thickening of the Axial Zone results from motion on thrust surfaces that join a basal detachment at the Moho¹⁴. Finally, using an inhomogeneous strain model (Fig. 2*d*), the superficial thrusts are considered to die out at depth in an area of strong ductile deformation; in this model, the offset of the Moho results essentially from a Cretaceous asymmetric tectonic event preceding Cenozoic thickening^{1,3,15}.

In order to constrain the deep structure of the Pyrenees, the main aims of the ECORS Pyrenees survey were to understand: (1) the geometry of the Northern Frontal Thrust and its relationship with the NPFZ; (2) the geometry of the northern Cretaceous basin, partly hidden by the northward thrusting; (3) the geometry of the southward-translated South Pyrenean units; (4) the geometry of the roof of the Hercynian basement and of the deep reflectors within it; and (5) the geometry of the Moho discontinuity. The results of the survey are shown in Fig. 3.

The entire crust shows well-defined reflectors with a general fan-shaped geometry (Fig. 3*a, b*). If we exclude the central part of the section (the Axial Zone), where southward- and northward-dipping reflectors interfere, the northern and southern forelands show a very clearly layered crust.

Near the surface, from south to north, the Ebro, Tremp and Aquitanian basins show continuous and highly energetic reflectors which represent Mesozoic and Cenozoic materials locally involved in Pyrenean deformation (Fig. 4*a*). At such places, usually below the major thrust structures (Sierras Marginales and North Pyrenean Frontal Thrust), the quality of deeper reflectors is affected by surface complexity. The well defined lower limit of the sedimentary succession dips on both sides towards the central part of the fold belt.

In the deepest part of the crust of the Iberian plate, a well layered domain starts between 10 and 12 s two-way travel time, and dips gently and progressively towards the central Axial Zone where it reaches a depth of 20 s. Its northward extension is questionable, because of the lack of processed data below 20 s. The base of this zone is interpreted as the Moho discontinuity (Fig. 4*c*). Below the Aquitanian basin, the layered lower crust thicker and shallower: it is located between 7 and 11 s and

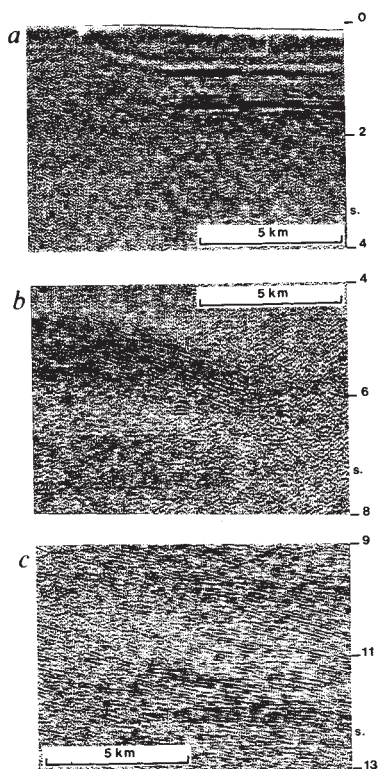


Fig. 4 Unmigrated seismic data showing the main seismic facies. *a*, Superficial continuous and planar reflectors due to sedimentary material and characterized by their high frequency (~ 25 Hz). *b*, Middle-crustal reflectors (medium-frequency): these are discontinuous, tightly spaced, gently dipping and generally connected to more horizontal reflectors. *c*, Deep reflectors at the base of the crust: these are energetic, discontinuous and numerous but not well aligned; their frequency is low (8–12 Hz). The seismic fabrics defined here were among those used to construct the cross-section in Fig. 3*b*.

dips gently to disappear below the Axial Zone, where the Moho cannot be identified as a single and continuous reflector.

The middle crust has the same seismic characteristics everywhere, even though it is thicker in the Iberian plate. Discontinuous areas of concentrated reflectors can be observed, mainly dipping northward (Fig. 4*b*). Although the crust of the Iberian side is thicker than that on the European side, the middle crust has similar seismic characteristics on both sides. In particular, discontinuous areas of concentrated reflectors are observed to dip northward on both sides (Fig. 3*a*, 3*b*). The Axial Zone in its upper and middle parts is bounded by reflectors dipping southwards in the north and northwards in the south, these being the expression of overthrusting towards the forelands (Fig. 3).

On both sides of the Axial Zone, the shallow seismic data can be unambiguously correlated with geological data (Fig. 3*c*). The seismic survey yields new constraints on the continuity, amplitude and geometry of the major thrust zones of the Pyrenean chain, which are summarized below.

Southern flank. One of the major results of the seismic profile is the observation of major northward-dipping reflectors within the Axial Zone, connected to the décollement of the Tresp basin and emerging in the Southern Frontal Thrust zone (Montsec and Sierras Marginales). The amplitude of displacement can be estimated using drill-hole data; it is known that Mesozoic and mainly Cenozoic series can be followed at least below the San Corneli hole (Fig. 3*a*). Other important results are that the thin-skinned tectonic style of the southern Pyrenean units is now well documented (Fig. 3*c*); the basement below the Southern Pyrenean Frontal Thrust is not involved in the Eocene (Pyrenean) deformation. Finally, the presence of small Permo-Triassic half-grabens below the Ebro and Tresp basins can be

assumed and is confirmed by oil drilling in these areas (not shown on figures).

North Pyrenean Zone. Here, the geometry of the northward-directed frontal thrust implies a large reactivation of the Hercynian basement in the Arize and Trois Seigneurs massifs, and the presence of Mesozoic deposits underneath these units. As on the southern flank, half-grabens fed by Permo-Triassic materials do occur in the Aquitanian foreland (not represented on figures). In the same area, northward-dipping reflectors, observed in the basement and not affecting the base of the sedimentary cover, were probably not strongly reactivated during the Pyrenean orogeny. They may represent either compressive Hercynian structures or younger, low-angle pre-Pyrenean fault zones.

North Pyrenean Fault Zone. The exact geometry of the NPFZ at depth is one of the key problems. Any interpretation must integrate the nature and origin of two kinds of reflector located immediately beneath the surface trace of the fault: the first and shallower group (4–7 s TWTT) being southward-dipping, the second, deeper group (14–20 s) being northward-dipping.

Figure 2*e* presents one of the models supported by the ECORS profile data. In this model, the shallower southward-dipping reflectors belong to the southern border of the European plate; the deeper ones correspond to the northern border of the Iberian plate. This model implies a strong deformation of the NPFZ and the indentation of the Iberian material by the European lower crust.

Despite the difficulty of the operation and the problems of data acquisition in the field, the quality of the obtained seismic section has allowed us to address some key problems concerning the Pyrenees and has provided useful constraints. The great difference in crustal thickness between the Iberian and European plates is confirmed: only the Iberian crust has been significantly thickened, and we believe that this results from the stacking of thrust sheets in the Axial Zone.

Finally, our data present difficulties for some of the previous models. The southward subduction hypothesis (Fig. 2*a*) is now difficult to support because of the geometry of the deeper reflectors beneath the Axial Zone (compare Fig. 3*a*). The model of a large, gently dipping sole thrust cutting the Pyrenean domain and European crust beneath the Aquitanian basin (Fig. 2*b*) also appears to be unrealistic; the pattern of reflectors in the middle crust and the attitude of the Moho beneath the Aquitanian basin seem to be incompatible with this hypothesis. This kind of model is acceptable, however, if there is a steeper thrust offsetting the Moho beneath the NPFZ, as in Fig. 2*e*. The thick-skinned tectonic model (Fig. 2*c*) does not explain the contrast in thickness between the Iberian and European crustal segments, and the pure vertical-flower-structure model (Fig. 2*d*) minimizes the importance of crustal-scale thrusts.

We emphasize the preliminary nature of the interpretation presented here; to refine the model further will require a consideration of new geophysical data (velocity models and gravity), on which work is in progress.

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Small-scale heterogeneities in depleted mantle sources: near-ridge seamount lava geochemistry and implications for mid-ocean-ridge magmatic processes

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The Lamont seamounts form a chain of five volcanoes extending northwestward from the flanks of the East Pacific Rise (EPR) near 10° N (Fig. 1). They have been investigated using Sea MARC I and Sea Beam sonar¹ and most recently with the submersible *Alvin*². During the most recent field programme we collected precisely located samples from stratigraphic sections on caldera and crater walls of each volcano and from the adjacent EPR. Major objectives of this programme were to determine whether the seamounts and the nearby ridge crest shared common mantle sources and to elucidate off-axis magma storage and ascent processes. The chemical compositions of Lamont seamount lavas, which include some extremely primitive basalts, indicate that each seamount was fed from one or more closely spaced mantle sources with depleted chemical characteristics. The geochemical and

isotopic data imply that there is no direct magmatic link between Lamont seamount and EPR accretionary magma sources. Lateral distances between individual seamounts, as well as those between the EPR axis and cones at the base of Sasha seamount are small (Fig. 1), leading us to the conclusion that chemical heterogeneities in depleted mantle sources exist on a very small scale (<5 km).

All lavas recovered from the Lamont seamounts (including smaller cones surrounding the main volcanoes) are light-rare-earth-element (LREE)-depleted tholeiitic basalts. No enriched (E)-type mid-ocean-ridge basalt (MORB) or alkalic rocks were found. We analysed 176 volcanic glasses for major elements by electron microprobe, and over 50 lavas for trace- and rare-earth elements. We also analysed a group of representative basalts from these volcanoes for Sr and Pb isotopes.

The lavas typically contain unzoned olivine and plagioclase as phenocrysts, although plagioclase in microlitic interiors may contain sodic rims. Clinopyroxene is absent in most lavas, and is present as microphenocrysts only within a few of the most evolved samples. Xenocrysts are similarly rare. This simple mineralogy implies very short residence times in magma chambers (if at all), with crystallization occurring at low to moderate pressures (<10 kbar) without extensive magma mixing or crustal assimilation.

The seamount glasses are on average more mafic than glasses from the EPR³ (Mg-number = 65.7 ± 2.8 against a mean of ~61) (Fig. 2). Many of the seamount and cone lavas are quite primitive (MgO to 9.74%, Mg-number to 72, Cr to 460 p.p.m., Ni to 240 p.p.m.), approximating those thought to be representative of primary mantle melts⁴. FeO, TiO₂ and Na₂O exhibit broadly linear increases with decreasing MgO concentration (9.75–6.85 wt%) whereas Al₂O₃ shows a marked decrease. CaO contents do not vary systematically but tend to be lower in the more evolved lavas. Concentrations of K₂O and P₂O₅ are low (<0.16 and 0.2 wt%, respectively), typical of N-type MORB. Most of the samples are silica-saturated but a few have minor amounts of quartz or nepheline in their norm.

The depleted nature of these lavas is exemplified by low abundances of incompatible elements (TiO₂ = 0.83–1.76 wt%,

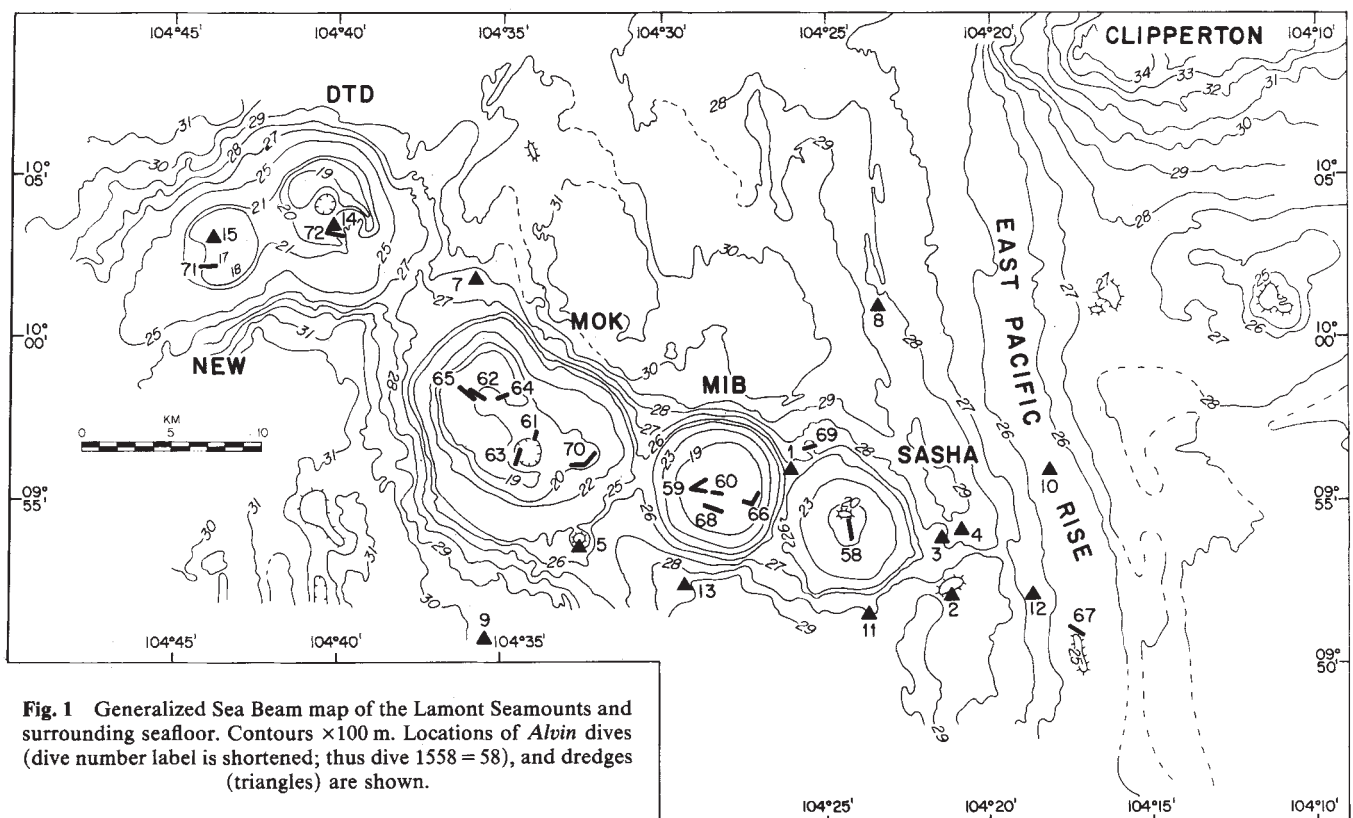


Fig. 1 Generalized Sea Beam map of the Lamont Seamounts and surrounding seafloor. Contours $\times 100$ m. Locations of *Alvin* dives (dive number label is shortened; thus dive 1558 = 58), and dredges (triangles) are shown.

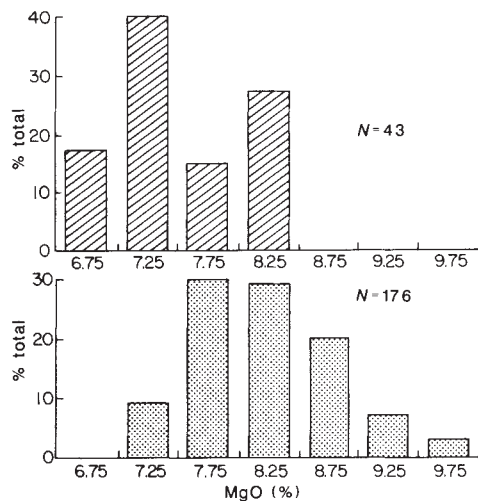


Fig. 2 Histogram showing relative MgO contents of lavas from the Lamont seamounts (bottom) compared with those from the adjacent EPR (top). The seamount lavas are overall much more primitive. EPR histogram includes data from Langmuir *et al.*³. Seamount data represent 176 analyses; EPR data represent 43 analyses.

La = 0.96–3.77 p.p.m., Hf = 1.19–2.69 p.p.m., Rb < 1 p.p.m.) and LREE-depleted chondrite-normalized REE patterns ($(La/Sm)_N = 0.25–0.57$). In general, Lamont seamount lavas are more depleted (even accounting for their more mafic compositions) and have lower incompatible element ratios than MORB from the adjacent EPR (Fig. 3). For example, as shown in Fig. 3a, three relatively mafic glasses from the axis of the EPR have significantly higher $(La/Sm)_N$ and TiO_2 contents than Lamont seamount basalts of comparable Mg-number.

Sr-isotopic ratios of Lamont seamount lavas range from 0.702224 to 0.702671 and correlate with Rb/Sr values (0.00048–0.0075) (Fig. 4). The total $^{87}Sr/^{86}Sr$ variation is about half the range measured in basalts from small, off-ridge seamounts that include transitional and E-type basalts⁵. N-type MORB from the EPR between 8° N and 21° N have a more restricted range of $^{87}Sr/^{86}Sr$ values but overlap with the higher ratios in the seamount glasses (Fig. 4)^{6,7}. The low Sr ratios in Lamont seamount glasses are comparable with the lowest values cited for MORB^{8,9}.

Pb-isotope ratios (Fig. 5) are similar to values reported for Pacific MORB and basalts from seamounts in the northeast Pacific^{5,8,10}. The Pb-isotope data plot along the same trends as other NE Pacific seamounts on Pb–Pb diagrams¹⁰. The range of ratios ($^{206}Pb/^{204}Pb = 17.954–19.572$, $^{207}Pb/^{204}Pb = 15.393–15.766$, $^{208}Pb/^{204}Pb = 37.299–38.854$) is significantly greater than that of MORB from the EPR, Juan de Fuca and Gorda ridges⁸. The most radiogenic value, from Sasha seamount, is even greater than those from NE Pacific seamounts influenced by enriched sources^{5,10}. Although the correlation among Pb isotopes is good there is only a weak correlation between $^{87}Sr/^{86}Sr$ and Pb isotopes. This fact has been noted by White *et al.*⁸ in their isotopic study of Pacific MORB. Clearly Pb isotopes in Lamont lavas reflect a significant degree of mantle heterogeneity within a very limited area.

In general, major element variations within each volcano may be explained by moderate (<30%) amounts of olivine plus plagioclase fractionation (in a ratio of 3:7) from a range of parental liquids differing slightly in Ca, Al, Na, Ti and the LREE at a given MgO content. This is exemplified by the multiple parallel fractionation trends of lavas on plots such as $(La/Sm)_N$ against Na_2O or TiO_2 (Fig. 3b). Variations in degrees of source partial melting cannot adequately explain the lack of correlation between the abundances of incompatible major and trace elements (for example, $(La/Sm)_N^{12,13}$ against $(Na_2O/Na_2O + CaO)$ or CaO/Al_2O_3). We therefore conclude that multiple parent

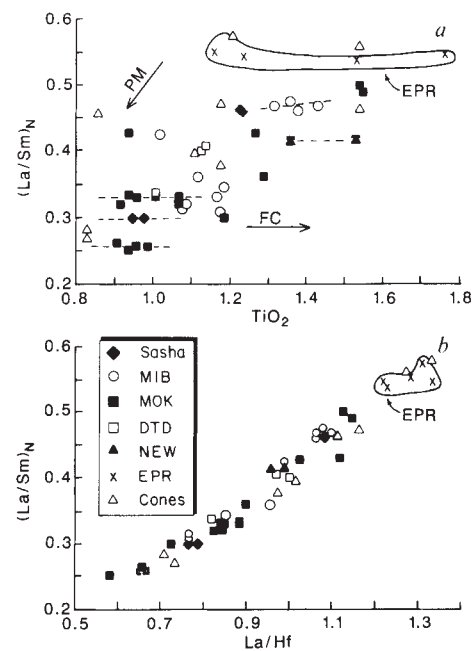


Fig. 3 a, $(La/Sm)_N$ against TiO_2 for Lamont seamounts and adjacent EPR lavas (outlined). FC arrow indicates low-slope fractionation paths, whereas PM arrow shows high-slope trends produced by varying the amount of partial melting of a mantle source. The short, subparallel fractionation paths (schematically shown by dashed lines) imply that multiple parental magmas are required to produce Lamont seamount lava compositions, even for different lavas from individual volcanoes. b, $(La/Sm)_N$ against La/Hf for Lamont seamounts and adjacent cone lavas and nearby EPR (outlined). Seamount and cone lavas contain a range of LREE abundances, EPR lavas are more enriched than most seamount and cone lavas.

sources are required for both the seamount chain as a whole and for individual seamounts.

Normative compositions of the most mafic Lamont seamount glasses are similar to experimental melts in equilibrium with spinel lherzolite at 9.0–9.5 kbar and 1,200–1,240 °C^{12,13}. This suggests that mantle equilibration of the parental lavas occurred in the transition zone between spinel and plagioclase lherzolite⁴, implying a mineralogically mixed source. Plagioclase and spinel lherzolite will melt differently, yielding magmas with different CaO/Al_2O_3 and $Na_2O/(Na_2O + CaO)$ ratios. Consequently, our estimate of magmatic equilibration at ~30 km helps to explain the discordance between the LREE and the less compatible major elements in Lamont seamount lavas. Because the more primitive seamount and cone magmas represent liquids that ascended quickly from the asthenosphere with little modification during crustal ascent, they serve as excellent probes for understanding near-ridge mantle source compositions and processes. In that respect, our depth estimate of 30 km may also reflect the pressure at which magma begins to be transported rapidly upwards by diking and crack propagation¹⁴.

Compared with lavas from the adjacent EPR, most Lamont seamount and cone lavas are more primitive, more depleted in incompatible elements and more chemically and isotopically heterogeneous. As discussed above, variations in LREE relative to major-elements and radiogenic isotopes cannot be ascribed solely to differences in the amount of partial melting of an N-type MORB source. Rather it appears that Lamont seamount lavas were derived from multiple, chemically heterogeneous mantle sources, each variably depleted in highly incompatible elements. The very close proximity of the Lamont seamounts to the EPR suggests that mantle source regions beneath spreading centres are likely to be more heterogeneous and chemically variable on a finer scale than previous sampling has implied.

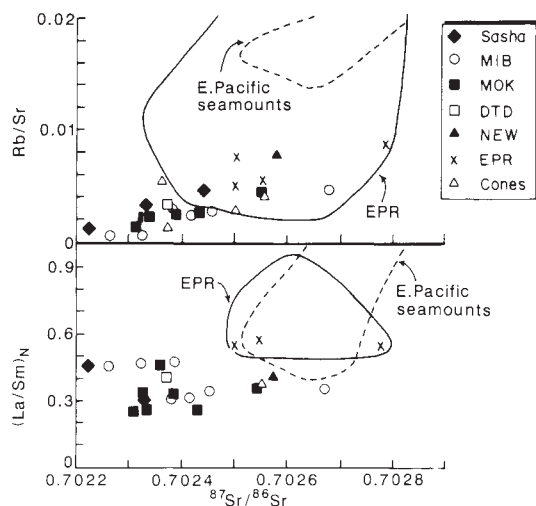


Fig. 4 $(\text{La}/\text{Sm})_N$ against $^{87}\text{Sr}/^{86}\text{Sr}$, and $^{87}\text{Sr}/^{86}\text{Sr}$ against Rb/Sr . Compositional fields for the northern EPR from the literature and from J. Bender and E. Klein (personal communication) are within the solid line. The compositional field for other eastern Pacific seamounts⁵ is within the dashed line.

Available geochemical data on ridge crest lavas^{3,7,15-21} point to a heterogeneous sub-ridge source, but the scale and magnitude of these heterogeneities are obscured by segregation, ascent and storage processes, all of which tend to homogenize chemical differences among distinct primary melt compositions and average out the isotopic signatures of mantle sources.

The primitive chemistry and diverse compositions of Lamont seamount lavas suggest that melts were produced in relatively small batches and that only minor amounts of differentiation occurred during ascent within each seamount's plumbing system. Furthermore, this probably occurred in ephemerally inflated portions of the shallow primary conduit rather than in long-lived shallow magma chambers. Beneath spreading centres, however, magma ascent paths are likely to be well established, and shallow magma chambers are more common and their replenishment is more frequent. Hence previous studies that have attempted to constrain the source characteristics of MORB lavas by sampling ridge crest lavas have probably only succeeded

in arriving at mean isotopic values and geochemical characteristics that represent a time-average of repeated magmatic events from an extremely heterogeneous mantle. The observed isotopic variation in the Lamont seamount lavas, together with the lack of covariation between trace and major elements, necessitates depleted sources that are both chemically and mineralogically heterogeneous on horizontal scales smaller than the diameter of single seamounts in the Lamont chain.

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Oxygen isotope dating of the Australian regolith

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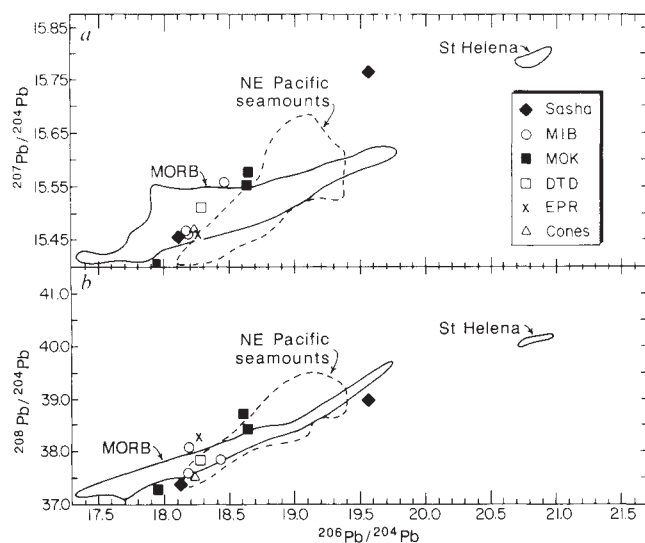
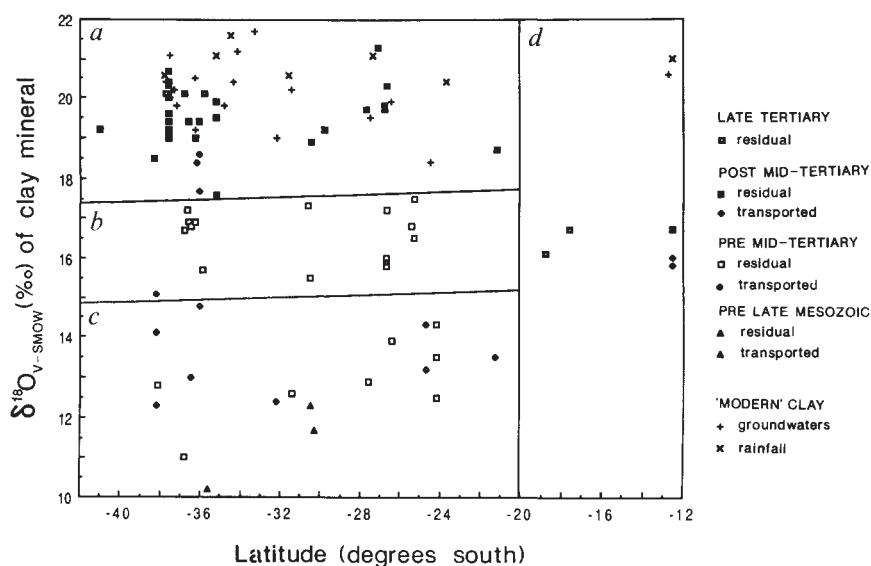


Fig. 5 *a*, $^{207}\text{Pb}/^{204}\text{Pb}$ against $^{206}\text{Pb}/^{204}\text{Pb}$ for Lamont seamount glasses relative to MORB and other NE Pacific seamounts. Data for fields shown in both *a* and *b* are from refs 10-11 and references therein. *b*, $^{208}\text{Pb}/^{204}\text{Pb}$ against $^{206}\text{Pb}/^{204}\text{Pb}$ for Lamont seamount glasses relative to MORB and other NE Pacific seamounts.

Australia has had a long and complex history of intense surficial weathering and this has led to the extensive development of laterites, bauxites and deeply weathered profiles of a variety of ages across the continent^{1,2}. A detailed knowledge of the age(s) of such weathering phenomena is of great importance for the reconstruction of Australia's post-Palaeozoic geomorphic and palaeoclimatic history. However, in many instances, absolute geochronological information is not obtainable using conventional techniques. Here we describe and evaluate a new method that can provide an estimate of the age of intensely weathered profiles based on the oxygen isotope composition of minerals developed by deep-weathering/lateritization processes. This method allows the definition of four ages of weathering in eastern Australia from the late Palaeozoic to the present and suggests that some profiles previously thought to be late Cretaceous or Tertiary in age may be of pre-late Mesozoic age. The oxygen isotope data indicate that the minerals used in this study have retained their original isotopic composition for periods of up to 250 million years, despite the continued presence of meteoric waters of progressively higher ^{18}O content during this interval.

Fig. 1 Oxygen isotope composition of Australian regolith minerals with respect to present latitude. The ages of the surficial clay samples have been assigned on geological evidence, usually an association of the regolith profile with dated sediments or volcanic units, and in some cases on palaeomagnetic evidence. Field *a*, post-mid Tertiary clays; field *b*, late Mesozoic to early Tertiary clays; field *c*, pre-late Mesozoic clays; field *d*, clays affected by the monsoonal climatic regime of northern Australia. The average deviation from the mean of replicate BrF_5 extractions for oxygen isotope analysis of the $<2.0 \mu\text{m}$ clay fraction of sampled regolith profiles is $\pm 0.25\%$, and all results are quoted relative to standard mean ocean water (SMOW). The plotted results have been corrected to an arbitrary altitude of 500 m above sea level, assuming an altitude effect of -0.2% per 100 m, and also for the presence of contaminating mineral phases (as determined by X-ray diffraction and electron microprobe bulk analyses). Hypothetical kaolinite compositions (labelled 'modern clay') calculated from published^{13,47-51} and unpublished (A. Chivas, I. Barnes and R. Evans) groundwater and rainfall data (adjusted to 500 m above sea level), assuming an oxygen isotope fractionation factor of 1.027 for the kaolinite-water system at surficial temperatures¹⁶.



Unlike parts of many continents in the Northern Hemisphere, where major Pleistocene glaciation resulted in the stripping of surface material from large areas of the landscape, Australia has remained essentially unglaciated since the early Permian, and in most areas erosion rates have remained low. This has meant that some land surfaces as old as Cambrian³, and weathered profiles, possibly as old as late Palaeozoic or early Mesozoic in age⁴, have been preserved in surface or near-surface exposures since their formation. Previous attempts to place constraints on the age of the Australian regolith have relied heavily on the identification of localities where such profiles are developed on, or overlain by, a sediment or igneous rock of known age⁴⁻⁷. This approach is often unsatisfactory in that it usually only provides a maximum or minimum age of weathering.

The palaeomagnetic dating of iron accumulations in weathered profiles, which relies on Australia's continental drift history, has the potential to provide good absolute estimates^{2,8,9}. However, the relationship between magnetization age and the age of formation of a clay mineral assemblage in a weathered profile remains ambiguous¹⁰.

The basis of the oxygen isotope dating technique proposed herein is that since rifting from Antarctica around 95 Myr ago^{11,12}, Australia has drifted rapidly north from a high-latitude position during the middle Cretaceous to its present position between 11° and 44°S. There is a strong correlation between the isotopic composition of meteoric waters and geographic factors such as latitude, altitude and continental position^{13,14}, and therefore, due to Australia's northward drift, the $\delta^{18}\text{O}$ of meteoric waters in Australia can be expected to have become progressively heavier since the mid-Cretaceous.

Minerals such as kaolinite and gibbsite are common in the Australian regolith¹⁵ and provide a useful recorder of this change because (1) their oxygen isotope fractionation factors are well known at surficial temperatures^{16,17}; (2) these minerals have been shown to form in isotopic equilibrium with surrounding groundwaters¹⁶; and (3) once oxygen of a given isotopic composition is incorporated into the silicate structure of these minerals it remains effectively bound and does not readily undergo isotopic exchange with later waters at surficial temperatures¹⁶⁻¹⁸. Most of the samples used in this study have been at the Earth's surface since their formation and none has been buried to depths great enough to lead to isotopic re-equilibration through diagenesis.

Thus, in Australia, these minerals can be expected to become increasingly depleted in ^{18}O with increasing age¹⁹ and by choosing samples from localities where there is at least one independent age constraint, it is possible to calibrate this change and then apply the method to profiles of unknown age.

Consideration of the conditions necessary for the development of highly leached weathering profiles suggests that coherent trends in the isotopic composition of clays from Australian regolith profiles, if present, would not be masked by excessive spatial and/or temporal variability. These conditions are that:

(1) The formation of laterites, bauxites and deep-weathering profiles require high rainfall and efficient leaching. Reaction rates are commonly aided by tropical or sub-tropical temperatures and preservation of the profile is assured by subdued topography¹⁹⁻²².

(2) The formation of such profiles, which in parts of Australia can be hundreds of metres thick^{2,15}, involve timescales of 10^4 – 10^7 yr^{2,9,23}. Hence only long-term gross variations in the isotopic composition of meteoric waters will be of importance in determining the isotopic composition of minerals in the profile.

(3) Evaporative effects, which can substantially modify meteoric water isotopic compositions, especially in arid zones^{13,24} will be negligible, due to the high rainfall conditions outlined above.

(4) Isotopic effects due to continental rainout will be substantially less than those calculated for temperate areas²⁵ and similar to those calculated for, for example the Amazon Basin²⁶, again due to the high rainfall conditions and the effects of transpiration by plants, which returns non-fractionated moisture to the atmosphere^{26,27}.

(5) Altitude effects will be similar to present values in humid climates of between -0.2 and -0.25% per 100 m for $\delta^{18}\text{O}$ (ref. 13).

The results presented in Fig. 1 represent $\delta^{18}\text{O}$ analyses of clays (most of which are kaolinite-rich) sampled from regolith profiles in Australia for which there is some independent geomorphic, stratigraphic or palaeomagnetic constraint on the age of weathering. The results are plotted with respect to present latitude and the areas delineated on Fig. 1 (fields *a* to *c*) have been drawn to separate isotopic results from clays of different age, and in the case of field *d*, geographic position.

A simple division of the sample ages into pre- and post-mid Tertiary groups is all that has been attempted because of the many potential sources of local variability in the isotopic compo-

sition of meteoric waters on a continent-wide scale, even over the long timescales envisaged for the formation of the profiles. The boundaries between sample groups *a*, *b* and *c* are constructed with a slope comparable to the latitude effect, which leads to increased $\delta^{18}\text{O}$ at lower latitudes. Analysis of multiple clay samples collected from the same locality has suggested a geological plus experimental error of $\sim \pm 0.5\%$ or less, in the $\delta^{18}\text{O}$ of clays from a given profile, independent of bedrock type. The majority of samples are from profiles developed on igneous and metamorphic rocks.

Isotopic results obtained from residual clays (collected *in situ* from regolith profiles) of post-mid Tertiary age have $\delta^{18}\text{O}$ values between $+17.5\%$ and $+10.0\%$. Compositions of $< +14$ to $+15\%$ field *d* (representing latitudes north of $\sim 20^\circ\text{S}$) which have anomalously low values. Meteoric waters in northern Australia are largely derived from monsoonal rain, which has been shown to be variably isotopically depleted compared to average equatorial waters²⁸, rendering northern Australia unsuitable for the strict application of this technique. A similar $\delta^{18}\text{O}$ depletion in modern soil carbonates has previously been suggested from areas of India that are subject to a monsoonal climate²⁹.

The post-mid Tertiary clays from field *a* have the highest measured $\delta^{18}\text{O}$ values, consistent with Australia's northward drift to low (warm) latitudes during this time, compared with the much higher latitudes it occupied for most of its post-Palaeozoic drift history³⁰. Late Tertiary residual clays have $\delta^{18}\text{O}$ compositions that are indistinguishable from all other post-mid Tertiary clays, supporting the conclusion that further subdivision of the sample groups is not feasible on a continent-wide scale.

Pre-mid Tertiary clays (Fig. 1 fields *b* and *c*) have $\delta^{18}\text{O}$ values between $+17.5\%$ and $+10.0\%$. Compositions of $< +14$ to $+15\%$ are geologically unreasonable considering the warm climate conditions prevailing in Australia during most of the late Mesozoic and early Tertiary^{31,32} and therefore isotopic values $< +14\%$ (which includes many samples that can only be shown on geological grounds to be older than mid-Tertiary) are thought to reflect weathering events during much earlier periods (early or mid Mesozoic?) when Australia was at a high latitude and global climate conditions were comparatively colder. Several workers have documented the existence of very old regolith profiles and land surfaces in Australia^{4,33,34} and support for the above hypothesis comes from three analyses of clays from profiles of stratigraphically demonstrable pre-mid Mesozoic age (Fig. 1, field *c*) which all have $\delta^{18}\text{O}$ values of between $+10.2$ and $+12.3\%$. Further support comes from the isotopic composition of Australian coals of early- and mid-Mesozoic age which have been shown to have depleted δD (and by implication $\delta^{18}\text{O}$) compositions, due to the high palaeolatitude at which they formed³⁵. Regolith profiles composed of depleted $\delta^{18}\text{O}$ clays ($< +14\%$) are widespread in Australia and it is possible that a much greater part of the modern landscape than previously recognized developed in the early- or mid-Mesozoic.

The $\delta^{18}\text{O}$ composition of transported (sedimentary) kaolinites (Fig. 1) reveals that the major source for many Tertiary clay deposits was the postulated early- to mid-Mesozoic depleted $\delta^{18}\text{O}$ regolith, and this continued to be so until at least the middle Miocene in some cases.

A comparison of the isotopic composition of hypothetical kaolinites in equilibrium with modern meteoric waters (Fig. 1) and the measured isotopic composition of clays from the Australian regolith indicates that the majority of post-mid Tertiary clays are in equilibrium with waters that are essentially identical to modern Australian meteoric waters. This is regardless of the fact that the ages of the post-mid Tertiary samples span 30–40 Myr, and suggests that the processes controlling the evolution of meteoric waters in Australia since it began drifting north cannot be simply related to progressively increasing temperature.

Factors such as global temperature fluctuations, changes in the patterns and intensity of atmospheric circulation, Australia's

latitude and orientation with respect to the latitudinal temperature gradient, and the development of the Antarctic ice-cap could all potentially affect the isotopic composition of meteoric waters in Australia. The comparatively warm Cretaceous polar climate³¹, coupled with steepening of latitudinal temperature gradients as circumpolar oceanic circulation became established^{36,37} would combine to reduce the temperature contrast experienced by Australia as it drifted north and if this were the case then it is likely that other factors associated with Australia's northward drift were also important in producing the isotopic differences between the pre- and post-mid Tertiary sample groups.

One such factor may be the northward movement of Australia from a zone of polar circulation, and increasingly into the influence of mid-latitude circulation. Gedzelman *et al.*³⁸ have shown that the δD (and therefore by implication $\delta^{18}\text{O}$) composition of stable cyclonic precipitation is depleted by $\sim 38\%$ relative to convective precipitation in northeastern North America. At present, rainfall in the region of polar circulation to the south of Australia is dominated by polar front cyclonic rainfall, whereas rainfall generated by convective instability is important in much of southern Australia³⁹. If such a relationship pertained in the past, then this, coupled with increasing temperature as a result of Australia's drift to warmer latitudes, would adequately account for the isotopic difference between the two age groups.

A fourth age of weathering can be identified by a distinct oxygen isotope composition ranging from $+4.4$ to $+9.0\%$, obtained from sedimentary kaolinites of early Permian age in the Gunnedah Basin, central eastern New South Wales. These samples have not been included in the accompanying figures as they are fully discussed elsewhere^{40,41}. The kaolinite is developed in thick clay-rock sequences in the Koogah and Willow Tree Formations⁴² and is thought to have resulted from the intense leaching of bedrock by waters at least partially derived from the deglaciation of the Australian continent in the Sakmarian–Artinskian⁴⁰. The isotopic composition of meteoric waters in equilibrium with these kaolinites ($\delta^{18}\text{O}$ less than -16%) is similar to those calculated by other workers for the early Permian in Australia^{43,44}, Antarctica⁴⁵, and India⁴⁶, which all formed part of the glaciated Gondwanan landmass at this time³⁰.

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Experimental vaporization and condensation of olivine solid solution

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Vaporization and condensation are the most important processes in the early solar nebula; both condensates and residues of vaporization may be related to the origin of meteoritic materials such as Ca-Al-rich inclusions, chondrules, their rims, and chondrite matrices. Although many thermodynamic calculations have been performed on the condensation of gases with compositions similar to that of the solar system¹⁻⁵, few condensation experiments have been carried out. We have vaporized olivine crystals and succeeded in condensing coarse-grained olivine, pyroxene, a silica mineral and metallic iron from a gas composed of olivine components and hydrogen at 1,200-500 °C at low pressures. Thus the most abundant phases in meteorites condense in the laboratory under conditions similar to those of the solar nebula.

The experiments were conducted in a high-vacuum chamber (35 cm long and 18 cm inside diameter). The furnace, which is located coaxially inside the vacuum chamber, is a W-wound alumina tube (5.0 cm long and 0.8 cm inside diameter)⁶. Temperature is measured with W-W₇₄Re₂₆ thermocouples, and is controlled to within ±5 °C. The sample is contained in a Mo capsule (3.5 mm long and 2.0 mm wide) with two orifices of 0.5 mm diameter. The capsule is hung at the hot spot of the furnace by a Mo wire. The temperature gradient of the Mo wire was previously measured; it decreases almost linearly from the hot spot (capsule) to the top of the furnace (for example, from 1,500 °C to 500 °C). Gas vaporized from the sample is released through the orifice of the capsule and recondenses on the Mo wire. The condensation temperature is assumed to be the same as the temperature of the Mo wire; however, this could include uncertainties of ±100 °C or more. The present furnace design is fundamentally different from those of previous condensation experiments (see for example refs 7-9), in which the starting materials were heated instantaneously by a laser beam or shock-wave, or in a solar furnace, and even the vaporization temperatures were not precisely known in most cases. It is important that in the present experiments both starting materials and gas

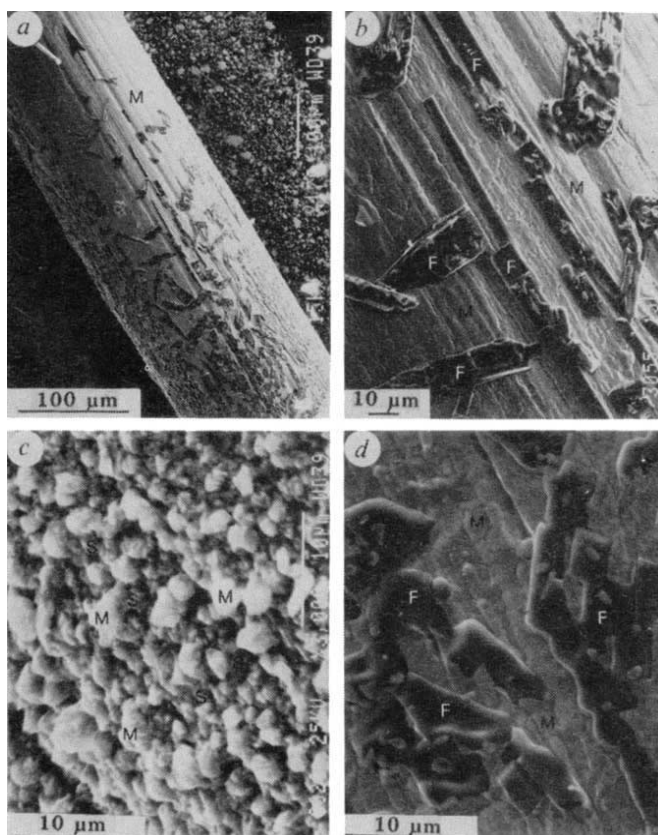


Fig. 1 Scanning electron micrographs of condensates at 3×10^{-9} bar total pressure. **a**, Forsterite condensed on the Mo wire (M) at about 1,200-1,150 °C. The direction of increasing temperature is shown by an arrow. Starting material (OL-2) was heated at 1,600 °C for 480 min (run number 3054). **b**, Enlarged view of **a**. Forsterite (F) elongates along the wire. **c**, Condensates formed in the middle portion (about 1,000 °C) of the Mo wire. Silicates (S) (dark) are mixtures of fine-grained iron-bearing pyroxene and tridymite. Molybdenum globules (M) (light) are present elsewhere. Starting material (OL-1) was heated at 1,550 °C for 1,440 min (run number 2972). **d**, Forsterite at the highest temperature portion of the wire in the same experiment as **c**. Forsterite crystals are platy, in contrast to those formed more rapidly (**a**).

are heated to known temperatures by radiation and conduction, and condensation starts from high temperatures. In contrast, gases in the previous studies were quenched, which resulted in the condensation of very fine grained or amorphous materials; the condensation temperatures were only roughly estimated.

The total pressure of the chamber was kept at 3×10^{-9} bar by bleeding H₂ gas into the chamber. Although the oxygen fugacity was not directly measured, it may be near the quartz-iron-fayalite buffer at least at low temperatures, because metallic iron, iron-rich olivine, and SiO₂ condensed on the low-temperature part of the Mo wire. Powders of olivine (1 to 30 μm) were used as starting materials to provide large surface areas. Two different starting materials were used: OL-1 (Fo₈₄), a phenocryst from basalt from the Miyakejima volcano, and OL-2 (Fo₉₂), a megacryst from San Carlos. Starting materials were heated for 6-72 h at 1,600, 1,550, 1,500 and 1,450 °C, which are between the calculated vaporous and solidus temperatures for olivine¹⁰. After the experiments, condensates on the wire and residues in the container were examined with a scanning electron microscope (SEM) equipped with an energy dispersive spectrometer (EDS) for quantitative analysis.

The fraction of the starting materials vaporized varied from ~40 to 10 wt%, depending on experimental temperature and duration. The residue after the experiments was stoichiometric olivine (Table 1), indicating that olivine vaporized 'congruently',

Table 1 Representative chemical compositions of the surface of residual olivines and forsterites condensed at the highest temperature portion of the wire (EDS analysis)

Run number	Residue		Condensate*	
	3,043	3,082	2,972	2,972
SiO ₂	42.31	40.76	39.09	39.68
FeO	3.84	10.72	0.63	—
MgO	53.73	48.12	50.80	52.29
CaO†	—	—	0.56	0.79
Total	99.88	99.60	91.08	92.76
Si	1.007	1.005	1.008	1.002
Fe	0.076	0.221	0.014	—
Mg	1.907	1.769	1.953	1.969
Ca	—	—	0.017	0.021
Sum	2.990	2.995	2.992	2.992
Fo	96.2	88.9	99.3	100.0

Treatment for run no. 3,043: 1,550 °C, 360 min; no. 3,082: 1,450 °C, 360 min; no. 2,972: 1,550 °C, 1,440 min.

* Low totals for analyses are due to the rough surface of the condensates.

† CaO is primarily contained in starting material, OL-1.

in accord with our previous results¹⁰. Strictly speaking, however, the observed vaporization was not congruent, because the Fe/Mg ratios of vapour and residual olivine are different. Average surface composition of the residual olivine varied from Fo_{97.5} at 1,600 °C to Fo₉₂ at 1,450 °C. These compositions suggest a magnesian solidus and an iron-rich vaporous in the olivine solid solution system, at least in the experimental temperature range.

The condensates show wide variations in grain size, phase assemblage, and in composition, depending on condensation temperature. In the run made at 1,550 °C for 480 min, rectangular forsterite crystals about 50 µm long and 10 µm in width condensed on the portion of the Mo wire at the highest temperature (~1,200–1,150 °C) (Fig. 1a, b). In an experiment of longer duration, the forsterite is platy (Fig. 1d). The condensates formed at lower temperatures are rounded and small-grained (Fig. 1c), and are morphologically similar, regardless of the experiment duration.

The temperature range where forsterite condenses is very narrow, and enstatite condenses at lower temperatures. Condensates at much lower temperatures are mixtures of iron-bearing pyroxene and silica. A transmission electron microscope study shows that pyroxene in the condensates at intermediate temperature is polysynthetically twinned clinopyroxene, probably inverted from protopyroxene (pyroxene hereafter), and that the co-existing silica mineral is tridymite. The lowest-temperature (about 500 °C) condensates are composed mainly of iron-rich olivine with lesser amounts of a silica mineral and metallic iron. The silica polymorph in this case has not yet been determined.

Forsterite condensed at the highest-temperature portion of the wire is euhedral, uniform in composition, and stoichiometric (Table 1), and no other phases accompany it. This strongly suggests that crystalline forsterite condensed directly from the gas, and is not a recrystallization product from a primarily condensed amorphous material. The lower temperature condensates, however, are mixtures of two or more phases, and some are subhedral to anhedral, so that it is not certain whether they directly condensed as crystalline materials or recrystallized from amorphous material. However, their morphology of protruding crystals is difficult to explain by recrystallization of amorphous materials. Thus all the minerals in the present experiments may have condensed directly from the gas as crystalline materials.

Figure 2a shows the compositional variations of the condensates formed by heating OL-1 at 1,550 °C and at 1,500 °C for 480 min, and those formed by heating OL-2 at 1,600 °C for

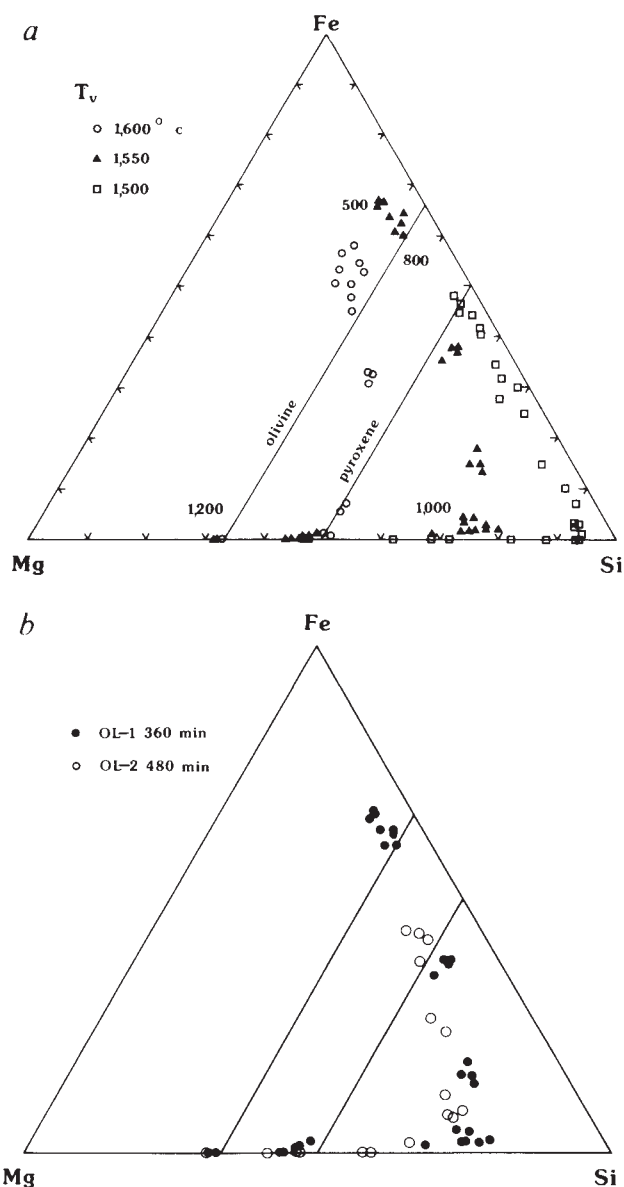


Fig. 2 a, Changes with temperature of the bulk chemical compositions of condensates for three different vaporization temperatures in the Mg-Si-Fe system (mole %). Total pressure of the chamber is kept at 3×10^{-9} bar. Numbers in the figure indicate approximate condensation temperatures (°C). b, Compositional changes of the condensates formed by two different starting materials at the same temperature (1,550 °C).

360 min. The highest temperature condensate is pure forsterite (Table 1). With decreasing temperature, an extreme enrichment in Si, with only a slight enrichment in Fe, is observed, and the condensates formed at lower temperatures are enriched in Fe with a decrease in Si. The composition of the condensates formed at the lowest temperature lies slightly on the Fe-rich side of the olivine join.

The low-temperature condensates have constant compositions and lie close to the olivine join. This suggests that the low temperature condensates are quench products from the gas: that is, the composition of the low temperature condensates may represent that of the gas vaporized from olivine of the starting material.

Experiments made at different temperatures show different compositional variations (Fig. 2a). The higher the vaporization temperature, the more the condensates are enriched in Mg and depleted in Si. Although the Mg/(Mg + Fe) ratio of the lowest

temperature condensates and the degree of SiO_2 enrichment are different for different vaporization temperatures, the highest temperature of condensation, the temperature of maximum enrichment in SiO_2 , and the $(\text{Mg} + \text{Fe})/\text{Si}$ ratio of the lowest temperature condensates are quite similar. Experiments made with different starting materials at the same temperature gave nearly the same results (Fig. 2b). These results demonstrate that the composition of the gas changes with vaporization temperature, but it is independent of the composition of the starting material.

Variations in composition and assemblage of the condensing phases with temperature can be understood in terms of volatility. The order of condensation with decreasing temperature from forsterite, through enstatite and silica, and further to metallic iron, is identical to that of increasing volatility as determined from the vaporization experiments¹⁰. The vapour pressures of olivine and pyroxene solid solutions are not known. In the present experiments, pyroxene of intermediate composition appeared at lower temperatures than silica, and iron-rich olivine appeared at lower temperatures than intermediate pyroxene. If the relationship mentioned above is applicable, the vapour pressure of iron-rich olivine may be higher than that of intermediate pyroxene.

Partial pressures of the condensing species such as MgO and SiO in the gas can be calculated from the equilibrium vapour pressures of the condensing phases (forsterite and enstatite)¹⁰, and the total pressure and condensation temperature of these minerals in the present experiments. Assuming that the total pressure is constant throughout the chamber and equilibrium condensation takes place, the partial pressure of MgO is calcu-

lated to be about 2×10^{-10} bar under the conditions where forsterite condenses (for example, $1,200^\circ\text{C}$) at 3×10^{-9} bar total pressure.

As demonstrated by the present experiments, vapour composed of olivine components and hydrogen condensed forsterite, enstatite, silica, intermediate pyroxene, iron-rich olivine, and metallic iron. The coexistence of magnesian olivine and a silica mineral, and the presence of olivine with compositions ranging from Fo_{100} to Fo_{10} in the matrices of chondrites¹¹, can be understood as resulting from the partial vaporization and recondensation of chondritic materials rich in an olivine component at different vaporization temperatures and at different degrees of undercooling or cooling rates. 'Wark-Lovering rims' on Ca-Al-rich inclusions (multiple layers of Ca-Al-rich phases with variable Fe contents) and chondrule rims in ordinary and carbonaceous chondrites, which are rich in metal, sulphide and volatiles, may also be the products of partial vaporization and recondensation.

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A deep-sea sediment transport storm

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Photographs taken of the sea bottom since the 1960s suggest that sediments at great depth may be actively resuspended and redistributed¹. Further, it has been suspected that active resuspension/transport may be required to maintain elevated concentrations of particles in deep-sea nepheloid layers. But currents with sufficient energy to erode the bottom, and to maintain the particles in suspension, have not been observed concurrently with large concentrations of particles in the deep nepheloid layer²⁻⁴. The high-energy benthic boundary-layer experiment (HEBBLE) was designed to test the hypothesis that bed modifications can result from local erosion and deposition as modelled by simple one-dimensional local forcing mechanics⁵. We observed several 'storms' of high kinetic energy and near-bed flow associated with large concentrations of suspended sediment during the year-long deployments of moored instruments at the HEBBLE study site. These observations, at 4,880 m off the Nova Scotian Rise in the north-west Atlantic, indicate that large episodic events may suspend bottom sediments in areas well removed from coastal and shelf sources.

Studies of the HEBBLE area since 1981 have described and quantified the effects on the bed of what have been inferred to be strong, episodic, sediment-modifying events⁶⁻⁸. Radiometric dating of samples from boxcores indicated that the rates of sediment deposition, and reworking by organisms, are exceptionally high, comparable even to river deltas⁷. Bedforms associ-

ated with strong current action, such as longitudinal ripples, have been dated and shown to form on the timescale of weeks⁹. Transmissometer profiles, *in situ* size analyses, photographs and water samples have revealed a strikingly high temporal and spatial variability in the concentration and composition of suspended material^{6,10,11}. This circumstantial evidence implied that sediment transport at this site occurred during episodic events of large magnitude 'storms' relative to the background conditions. Long-term monitoring of the bottom boundary layer and sediment response was undertaken to test this model of erosional and depositional 'storms'. Several significant events were observed over a period of one year. The strongest event is described here.

The active Nova Scotian Rise area ($40^\circ 26.5' \text{ N}$ $62^\circ 21.7' \text{ W}$) was selected for HEBBLE experiments because the bathymetry and surface composition of the sediments are laterally homogeneous and provide a 'best site' to test one-dimensional models. The large-scale topography is dominated by the continental rise which, in this area, has a slope of 1/200. There are minor undulations of 2-3 m in the vertical and 300-600 m in the horizontal dimensions. The bottom is composed of burrowed consolidated silt and clay. Photographs (Fig. 1) showed that the surface of the sediment is extensively tracked and worked by a variety of benthic biota¹². The logarithmic velocity profile roughness scale is 0.5-1 cm ($z_0 = 0.015 \text{ cm}$)¹³. This confirms the visual impression from photographs that the dominant surface roughness is set by the mounds, burrows and foraging tracks of the biota.

Three sequential deployments of bottom-landing, instrumental tripods were made, from September 1985 to September 1986, to quantify the strength, duration and variety of bottom modifying 'storms'. Two of the tripods were arrayed with benthic acoustic stress sensors (BASS) to measure turbulent velocity vectors near the bed (0.38, 0.73, 1.32 and 5.02 m above bottom (mab)¹⁴. All six components of turbulent stress and energy, and mean velocity vectors, were measured by sampling at a 2-Hz rate and averaging over 30-min intervals¹³. Suspended sediment concentrations, calibrated at $1,208 \mu\text{g l}^{-1}$ per metre of depth¹⁵,

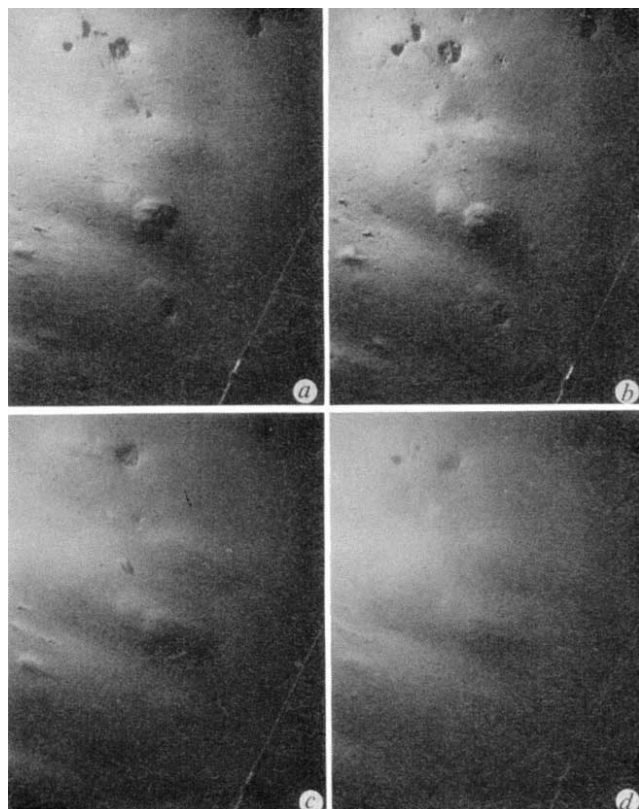


Fig. 1 *a*, 16 May: After a bottom-modifying event the surface was relatively smooth. *b*, 12 June: 3 weeks later extensive tracking and bottom modification occurred during the low flow period. *c*, 22 June: After the major storm, many of the burrows were filled in and crag and tail streamlining features grew. *d*, 5 July: After a period of relatively low suspended load, but moderate currents, there was little or no net erosion/deposition. Extensive bed modification in the form of elongating tails in the lee of bumps occurred. The photographs were taken from the stationary platform of the BASS tripod during the May-July 1986 deployment. The field of view is 2 m $\times$ 2.6 m. The camera was 4.4 mab.

were measured with Sea Tech transmissometers positioned at 2.20 and 4.50 mab. Thermistors and a camera were also arrayed on the tripod platform. Other instruments on the tripod, but not discussed here, were: an acoustic back-scatter sensor, to measure vertical structure of suspended sediment¹⁶, a laser doppler velocimeter, to measure fine-scale near bed turbulence¹⁷, and a remote optical settling tube, to measure the size distribution of suspended sediments¹⁸.

Four major events with suspended sediment concentrations exceeding $2,000 \mu\text{g l}^{-1}$ and durations of 2–5 days were observed during the year. Background suspended sediment concentrations during quiescent periods were $<500 \mu\text{g l}^{-1}$. The BASS data for the event during which light attenuation was the greatest (14–19 June) is shown in Fig. 2. The BASS at 4 mab provided 30-min averages of the velocity vector, the direction and speed of which is shown in Fig. 2*a* and *b*. The turbulent kinetic energy averaged from 2-Hz data over 30-min intervals from the BASS at 0.73 mab is presented in Fig. 2*e*. The kinetic energy is roughly proportional to the mean speed squared. However, changes in and anisotropy of bottom roughness caused variations with time and flow direction from the quadratic relation. This effectively varying drag coefficient necessitates independent measures of both turbulent and mean velocity scales. Light attenuation at 2.2 mab, converted to suspended mass concentration is plotted in Fig. 2*c*. Throughout the deployment the variation between transmissometers at 2.2 mab and 4.5 mab was $<1\%$. Temperature records from 4 mab (Fig. 2*d*) can be an indicator of water-mass changes and entrainment into the variable depth mixed layer¹⁸.

The June event was exceptional during the year-long deployment because it carried the largest load of suspended sediment. For the month preceding the event the currents were quite weak and fluctuated predominantly about the south-west. Internal tides caused maximum velocities of 10 cm s^{-1} . The currents veered to the south-east one week before the 15 June maximum and increased the average speed to 12 cm s^{-1} . The currents turned 90° towards the south-west, increased to the maximum of 22 cm s^{-1} on 15 June and had a tidal signal of only $\pm 1.5 \text{ cm s}^{-1}$. The currents increased by a factor of two over the non-erosional period while the turbulent stresses increased by nearly a factor of three. The very rapid rise in suspended sediment concentration on 14 June indicates that critical erosion was surpassed as twice the turbulent kinetic energy, q^2 , exceeded $9 \text{ cm}^2 \text{ s}^{-2}$. Peak suspended sediment concentration of $5,000 \mu\text{g l}^{-1}$, 2 m above bottom, was reached after 3 days of increasing currents and $\sim 10 \text{ h}$ after maximum current velocity. The peak sediment concentration coincided with the peak turbulent energy, and lagged the velocity peak by 10 h.

Total sediment transport in the nepheloid layer is calculated from the integrated concentration through the layer times the depth of the layer and velocity. The depth of locally generated nepheloid layers is determined by the depth of the turbulent boundary layer when the sediment has had time to mix throughout the layer. The Ekman layer depth scales as $\kappa u_* f^{-1}$, where $\kappa = 0.40$ is von Karman's constant, $f = 0.943 \times 10^{-4} \text{ s}^{-1}$ is the Coriolis frequency, and u_* is the turbulent shear velocity scale^{19,20}. The turbulent shear velocity scale can be estimated from direct measures of the turbulent kinetic energy by, $u_*^2 = 0.15 q^2$, where q^2 is the sum of the velocity vector component variances or twice the turbulent kinetic energy²¹. For the peak kinetic energy of $9 \text{ cm}^2 \text{ s}^{-2}$ during the storm, u_* is 1.2 cm s^{-1} . The boundary-layer depth is 51 m. If the sediment particles are carried passively by turbulent motions, characterized by an eddy diffusivity, K , random walk theory gives the probable location after time T as $Z = (2KT)^{1/2} - Tw_f$ (w_f is the particle fall velocity)²². For small fall velocity, $w_f < 0.01 \text{ cm s}^{-1}$, and $K = \kappa u_* \delta / 2 = \kappa^2 u_*^2 / 2f$, the time taken to disperse to the height $\delta = 51 \text{ m}$ is $T = \delta^2 / 2K = \delta^2 / \kappa u_* \delta = 1/f = 10,600 \text{ s} = 3 \text{ h}$. Maximum concentrations are reached in $\sim 10 \text{ h}$, indicating that the development of suspended sediment profiles is source, rather than time, limited. The vertical distribution of suspended sediment depends strongly on the fall velocity of the particles in suspension. Data from the remote optical settling tube, used to measure *in situ* fall velocities, showed that sediments in suspension had fall velocity peaks at 5×10^{-4} and $2 \times 10^{-2} \text{ cm s}^{-1}$ (see ref. 18). During the June storm, large turbulent shear stress and small fall velocities resulted in almost no vertical structure of these size classes. Thus the total sediment in suspension was mainly a function of depth of the boundary layer, δ . The total suspended load is, therefore, $\sim 25,000 \mu\text{g cm}^{-2}$. Assuming that the suspended load is locally generated, the bed erosion required would be $\sim 1.0 \text{ mm}$ of highly porous sediment of relative density 0.25 g cm^{-3} . The mean velocity of 22 cm s^{-1} gives a horizontal transport of $1.98 \text{ kg cm}^{-1} \text{ h}^{-1}$.

The maximum concentration of $5,000 \mu\text{g l}^{-1}$ was maintained in suspension for only 6 h when q^2 was greater than $9 \text{ cm}^2 \text{ s}^{-2}$. During the following 4 days the sediment fell out of suspension more slowly than it was eroded, but still far faster than required for a fall velocity of 0.001 cm l^{-1} to clear the water. Either larger particles made up the storm cloud or flocculation is needed to explain the rapid clearing. The major depositional flow occurred during the 4 days after the peak when the flow was orientated towards west-south-west as part of an abyssal western boundary current or the cold filament current^{23–25}. After 19 June the stress was as low as during the prestorm period, but there was still $1,000 \mu\text{g l}^{-1}$ in suspension. This residue was the very slow settling clay aggregates whose clearing rate was determined by the rate of flocculation rather than the unflocculated Stokes fall velocity¹¹.

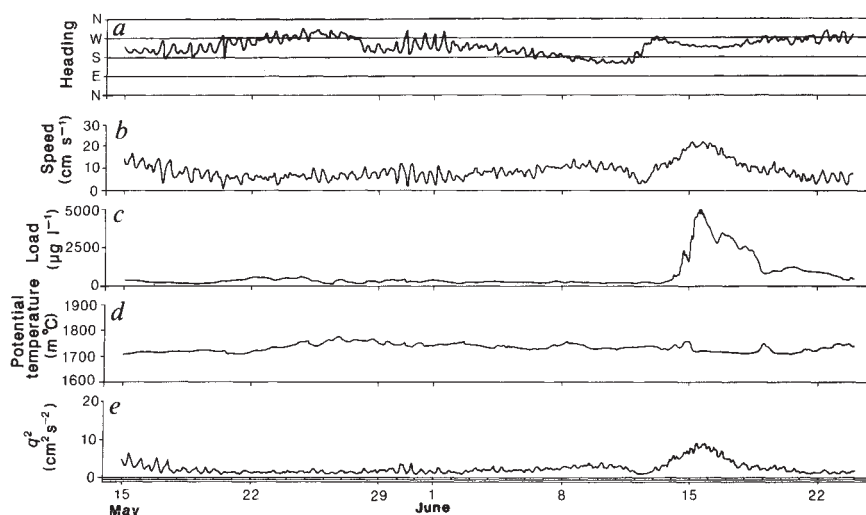


Fig. 2 *a, b*, Direction and speed of the 30-min average vector velocity measurements at 4.5 mab. *c*, Suspended sediment load at 2.2 mab. *d*, Potential temperature at 4 mab. *e*, Turbulent kinetic energy obtained from direct measures of the total velocity vector variance from 30-min averages of 2-Hz measurements at 0.73 mab. Time series of data from the BASS tripod instruments for the low-flow period preceding the storm through the major suspension event 15 May–24 June 1986.

Photographs of the bottom show that significant changes in the surface sediment texture occur on timescales of days to weeks. During the periods of low flow, benthic fauna roughened the surface by tracking, burrowing and defecation. In addition, the motion of seaweed rolling across the bottom cut striations in the prestorm flow direction. These changes can affect the turbulent boundary layer by roughening the surface, thus causing increased surface drag. At the HEBBLE site the logarithmic profile roughness parameter, z_0 , can increase by a factor of five during periods of low flow over the value following a few days of strong bottom modifying flow¹³. Ageing and biological activities can also alter sediment adhesion and composition thus greatly affecting the critical erosion stress. These and other effects result in different responses of the bed under similar flow conditions resulting in a variety of 'sediment storms'.

A comparison of bottom photographs taken before and after the storm show changes in roughness caused by the storm and by biological activity. Sediments deposited under a current are less eroded by flows from similar directions due to the formation of crag and tail features which shelter easily eroded sediments in the lee of larger surface non-uniformities. Initial particle motion can be caused by biological activity, allowing bedload during low flow. These occasionally moving particles can be stopped in the lee of mounds and other small-scale inhomogeneities. Crag and tail features built up during low flow conditions can be seen in some of the photographs (Fig. 1*c, d*). Sediments prepared in this manner under the influence of south-east currents will be more easily eroded by flows from other angles, towards the south-west. At other times of the year when flow exceeded 22 cm s^{-1} the concentration of suspended matter did not exceed $1,000 \mu\text{g l}^{-1}$ of sediment. Before the June storm the sediments were 'preconditioned' for erosion by south-westward current because of the previous week of low velocity southeasterly flow and weeks of biological roughening. The rapid increase in velocity and change of direction more easily entrained material that was previously prepared for erosion.

Although the large storm of 15 June may have suspended a total of only 1.0 mm of sediment from the bottom, the bed modification involved much more sediment. The photographs show that the surface sediments were rearranged, the animal tracks smoothed out, burrows filled in, and crag and tail features developed. It is apparent from these photos that several millimetres of sediment were deposited in the lee of the animal mound in the centre of the photograph. The sharp ridge on this feature bears a close resemblance to the longitudinal ripples seen in previous photographs of the area⁹, although this ripple is far smaller. As with longitudinal ripples the axis of this ripple is aligned with the post-storm depositional flow. The pictures from days following the storm are quite cloudy, but it can be discerned that this small longitudinal ripple grew during the

week of deposition and major bed modification, following the peak storm stress.

The occurrence of large 'storms' in the deep sea has been directly demonstrated from data obtained from the long instrument deployments at the HEBBLE site. While many of the large suspended load events observed throughout the year may be clouds of fine suspended material advected into the area, the June event provides an example of the effects of a locally forced sediment transport storm. During the short duration of this storm the surface of the bed was significantly modified.

It has been suggested that the bottom nepheloid layer could be caused by distant sources or occasional sediment storms^{3,26,27}. The usually observed bottom nepheloid layer with $200\text{--}500 \mu\text{g l}^{-1}$ particle load could result from the occasional $5,000 \mu\text{g l}^{-1}$ storms occurring at a frequency of one or two per year. The signature of the nepheloid layer maintained in this manner should have considerable temporal and spatial variation as the recirculating North Atlantic bottom-water gyre experiences only a few localized storms per rotation. The possible occurrence of events throughout the gyre is, at this time, only speculation. As the HEBBLE study site is in an area of exceptional currents, but with fairly representative sediment types, one can expect that only a few storms per year appear in the body of the gyre away from the continental slope. The ability of these deep sea storms to maintain the nepheloid layer depends on the composition, fall velocity, flocculation rates, diffusion of the particles which make up the deep-sea nepheloid layer and the extent, duration and frequency of the storms. It seems reasonable to suggest that the occurrence of a few storms per year, which suspend concentrations of $>1,000 \mu\text{g l}^{-1}$ of sediment, as shown here, may be sufficient to account for most of the suspended load in the deep-sea nepheloid layer.

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Nitrate flux into the euphotic zone near Bermuda

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Throughout most of the year, there exists within the North Atlantic subtropical ocean mixed layer a ^3He excess which can only be attributed to a flux of tritiogenic ^3He from below. The upward flux required to support this excess can be computed as the convolution of the time series of ^3He excess and gas-exchange rate. This flux is quantitatively consistent with the long-term evolution of the main thermocline inventories of tritium and ^3He . Using the observed correlation of ^3He with nitrate within the upper main thermocline, I estimate the annually averaged upward flux of nitrate into the euphotic zone to be 0.6 ± 0.2 moles m^{-2} per yr. This flux is sufficient to support a new, non-regenerative primary productivity in excess of 3 moles carbon m^{-2} per yr.

Recently, two lines of evidence based on dissolved oxygen distributions have been used to argue for new (non-regenerative) production rates in oligotrophic waters of ~ 3 moles of carbon m^{-2} per yr (refs 1-6). Such estimates appear inconsistent with previous doctrine regarding new production in oligotrophic waters (ref. 2, compared with ref. 7). I feel confident of the higher estimates because they are based on a number of different approaches, and on two separate cycles: the photosynthetic production rate of oxygen within the euphotic zone¹⁻³, and the consumption rate of oxygen in the water column below⁴⁻⁶. Further, tracers offer the advantage over more 'instantaneous' and 'local' measurements such as bottle incubations, of providing a large space and long timescale average of what are probably sporadic and patchy processes.

However, the existence of such high productivity raises the question of how to transport sufficient nutrients into the euphotic zone to fuel this new production. ^{15}N -labelled uptake experiments in the tropics⁸ indicate sufficient fluxes do occur, and are consistent with reasonable vertical turbulent diffusivities; but vertical nitrate gradients within the subtropics (where the question of high productivity has been raised¹⁻⁶, are much weaker. Consider, for example, a typical vertical nitrate gradient in the Sargasso Sea upper main thermocline of $2-3 \times 10^{-5}$ moles m^{-4} . Using accepted values of diapycnal turbulent diffusivities ranging from 1 to $5 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$ (for example, refs 9-11) one estimates an upward flux of nitrate from 0.01 to 0.05 M m^{-2} per yr. This is an order of magnitude smaller than the flux required by the new high production levels. A more sophisticated approach considering microstructure yields similarly low fluxes¹². The concern, though, is that the above may not include potentially important pathways associated with isopycnal and mesoscale processes. Rather than attempt to compute the nitrate flux based on advective-diffusive modelling, or direct observation of mixing events, I make a more direct estimate of the nitrate flux by using the isotope ^3He as a flux gauge.

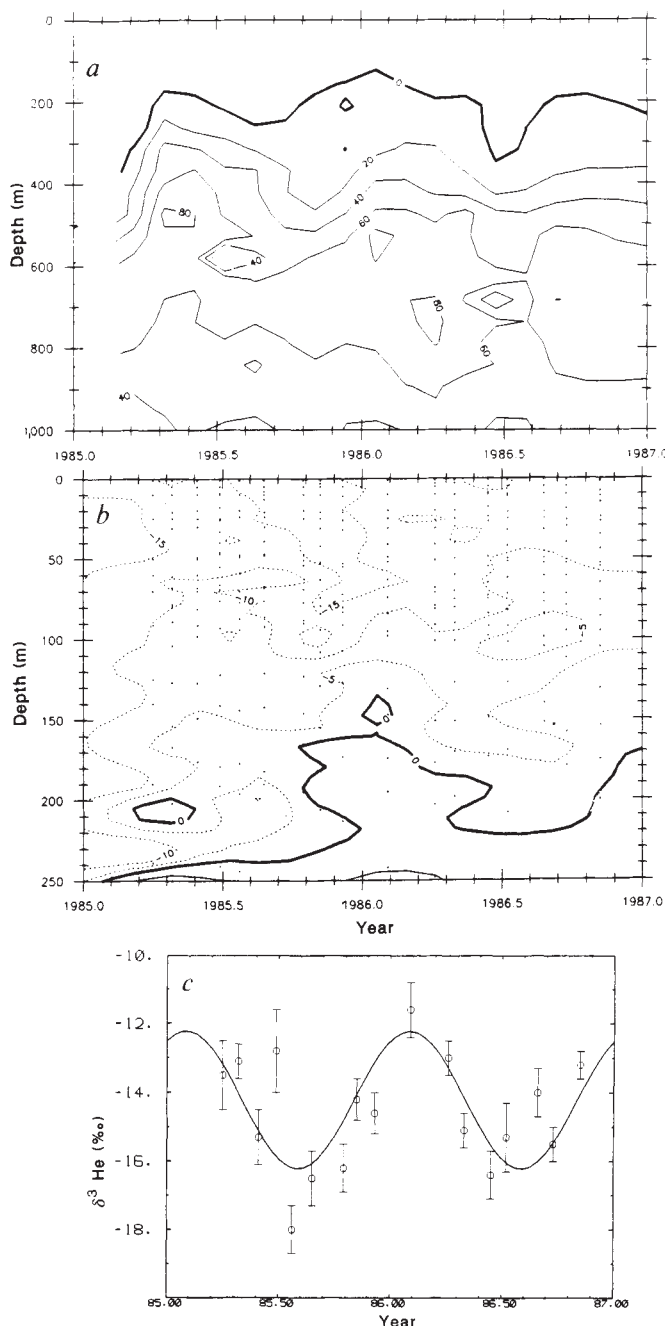


Fig. 1 The time history of $\delta(^3\text{He})$ against depth for 1985-86 near Bermuda: *a*, to a depth of 1,000 m; and *b*, to a depth of 250 m. Sample depths and times are indicated by dots in the latter. *c*, The mean mixed layer $\delta(^3\text{He})$ for 1985-86. Mixed-layer depth was determined from the temperature distribution. Uncertainties (error bars) are standard deviations of the means for each station. The sine wave (heavy line) is obtained from a weighted, nonlinear least squares regression of the data.

The penetration of nuclear weapons produced tritium (^3H , half-life 12.45 yr) has created an inventory of ^3He , its stable, inert daughter product within the main thermocline of the North Atlantic. This can be seen in Fig. 1 which is a two year time series of the helium isotope ratio anomaly (relative to atmospheric helium). This anomaly is defined by

$$\delta(^3\text{He}) = (R_x/R_a - 1) \times 1,000\%$$

where R_x and R_a are the sample and atmospheric isotope ratio ($^3\text{He}/^4\text{He}$) respectively. Measurement uncertainty is $\sim 1.5\%$. ^3He is slightly less soluble than its sister isotope (^4He), so that water in solubility equilibrium with the atmosphere is characterized

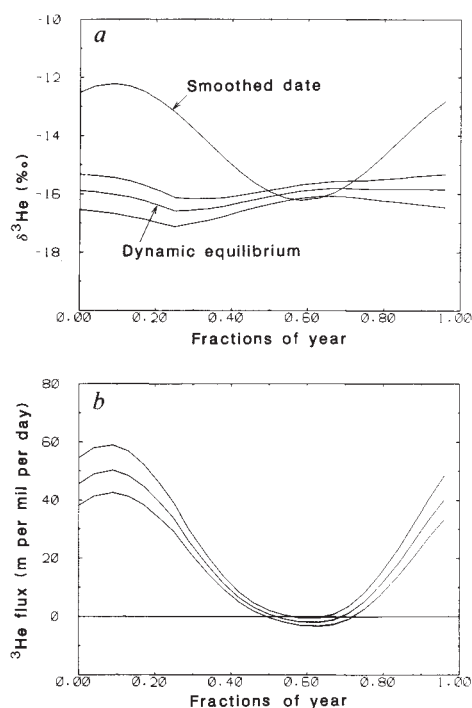


Fig. 2 *a*, Dynamic equilibrium $\delta^3\text{He}$ and its uncertainty (indicated by the lower solid lines) obtained using an upper ocean seasonal model incorporating air injection and calibrated using helium and neon saturation anomalies¹⁴. *b*, The estimated time history of ^3He flux (and its uncertainty) from the mixed layer. Note that this does not represent the timing of the entry of ^3He into the euphotic zone from below.

by an isotopic ratio anomaly of $\sim -17\%$. From the mixed layer, the helium isotope ratio anomaly increases from near-equilibrium values to a maximum at 700-m depth of $\sim 70\%$. The upper few hundred metres (Fig. 1b) show substantial temporal variability on approximately seasonal timescales, and in general the mixed layer appears to have an excess of ^3He over solubility. Particularly striking are the tendrils of ^3He reaching upward below the mixed layer even during the summer months. The mixed layer excess is more evident in Fig. 1c, which is a plot of the mean mixed layer $\delta^3\text{He}$ against time. The error bars represent one standard deviation of the means obtained, and include both the analytical uncertainty and the variability of the isotope ratio within the mixed layer. Variability within the mixed layer is significantly greater than analytical error. This variability must be the result of both horizontal and vertical shear-induced heterogeneity of the tracer as it is entrained into the mixed layer.

There is evidence of both a general annual cycle and a hint of an interannual trend. It is notable that the greatest ^3He excess appears to occur during the winter: deep convection harvests tritiogenic ^3He from the waters below, maintaining a large excess of the isotope in the mixed layer despite vigorous gas exchange. In the summer, little excess accrues, because stratification below the mixed layer presents a barrier to upward transport of this isotope. This does not mean that there is a cessation of upward transport of ^3He (and other properties) into the euphotic zone during the summer, but rather that this transport is not reflected in the mixed layer inventory above. Continuity requires that the flux of this isotope into the mixed layer must be equal to the flux into the euphotic zone on long enough (annual) timescales.

The existence of excess ^3He in the mixed layer means that there must be a flux of this isotope via gas exchange to the atmosphere. I show that only part of this flux can be supported by *in situ* production by tritium decay within the mixed layer. The bulk of the flux must come from below. I propose to use

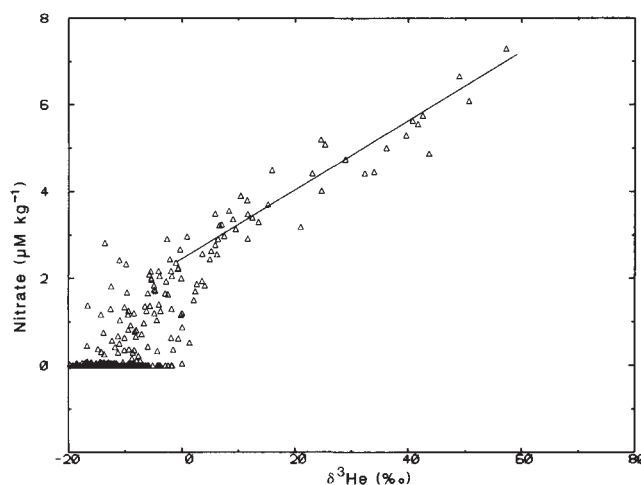


Fig. 3 Nitrate (in $\mu\text{M kg}^{-1}$) against $\delta^3\text{He}$ for the upper main thermocline for 1985–87. Note the substantial non-conservative behaviour of nitrate at low values due to biological utilization in the euphotic zone. The line corresponds to a linear regression of the deeper values. Slope used for ^3He –nitrate calculation was $0.080 \mu\text{M kg}^{-1} (\text{per mil})^{-1}$.

the observed ^3He excess along with gas-exchange rates to estimate this flux of ^3He into the mixed layer, and to use the observed relationship between this isotope and nitrate within the upper main thermocline to compute the corresponding upward flux of nitrate.

To begin, I fit a sine curve through the data (the heavy line in Fig. 1c). The choice of the sine curve is arbitrary, but offers the advantage of a closed annual cycle, and a tendency to conservative (lower) values in the winter months when gas exchange is high. Thus I am attempting to make a modest estimate of the requisite ^3He flux. The 'dynamic equilibrium' helium isotope ratio anomaly and its uncertainty (the lower lines in Fig. 2b) can be estimated using an upper ocean seasonal model^{3,13} incorporating air injection effects and constrained by observed helium and neon saturation anomalies¹⁴. The time series of ^3He excess is then the difference between observation (upper sine wave) and the equilibrium curve.

The climatological seasonal cycle in gas-exchange rates can be obtained using the wind speed against piston velocity relationship of Roether¹⁵ and the climatological seasonal wind speed record¹⁶. Here I use a broken line fit to Roether's data, with the parameters for low wind speed ($W < 4 \text{ m s}^{-1}$) of

$$V_p = 0.120 \times W$$

and for higher wind speed ($W > 4 \text{ m s}^{-1}$) of

$$V_p = -3.47 + 0.9995 \times W$$

where V_p is the gas-exchange piston velocity expressed in m per day, and W is the wind speed in m s^{-1} . Similar, if not greater exchange rates are obtained from other relations (for example, ref. 17). Regression statistics for the fit indicate an estimation error of $\sim 15\%$ over the range of interest. Our observations of the seasonal cycling of Ar in the upper ocean are consistent with these gas exchange rates (manuscript in preparation). To extend these gas-exchange rates to helium, I use a square root dependence on the Schmidt number¹⁵.

$$Sc = \nu / D$$

where ν is the kinematic viscosity of water and D is the molecular diffusivity of the gas in water. I have again selected a conservative path, because the choice of a steeper dependence on the Schmidt number, (as has been proposed in ref. 18) would result in larger estimated gas-exchange fluxes. Further, the use of climatological (averaged) winds in the calculation tends to underestimate the

average gas-exchange rate (and hence the gas flux) because of the overall nonlinear dependence of the gas-exchange rate on wind speed.

The resultant ^3He flux is the convolution of the excess ^3He record with the gas-exchange rate history (Fig. 2c), that is, the product of the piston velocity with the mixed-layer mean excess ^3He . It should be noted that this cycle represents the timing of the loss of this isotopic from the mixed layer and not the timing of its entry into the euphotic zone from below. Mass continuity demands that on the annual mean, the two must be equal; but the instantaneous value of the former is governed by the time-dependent processes governing mixed-layer evolution. The two-year mean ^3He flux thus computed is 21 ± 4 m per mil per day, where the uncertainty incorporates both uncertainties in the gas exchange rate and in the equilibrium helium isotope ratio anomaly. Using the mean mixed-layer tritium concentrations (~ 3 tritium units (TU) where 1 tritium unit = 1 tritium atom per 10^{18} H atoms), this number can be adjusted downward to account for *in situ* decay within the upper 100 m (2 ml per mil per day), giving a net annual mean flux of 19 ± 6 m per mil per day for ^3He .

This flux is supported by the 'mining' of tritiogenic ^3He from the main thermocline. Comparison of the 1986 profiles with tritium and ^3He data obtained from this station in 1977¹⁹, provides an estimate of the long-term changes in the inventory of these isotopes in the main thermocline. The equivalent rates of change in the tritium and ^3He inventories in the upper 700 m (above the oxygen minimum) are 12 and 4.5 m per mil per day respectively. The combined flux agrees well with the mixed-layer flux estimate. While precise agreement cannot be demanded (an exact local balance of these isotopes should not be expected: their entry and exit points are likely to be elsewhere within the subtropical gyre), it is encouraging that even approximate consistency is obtained.

Above the oxygen minimum, nitrate and ^3He are positively correlated within the upper main thermocline (Fig. 3). The reason is simply that as one goes deeper into the main thermocline, one encounters older water, hence greater nitrate and ^3He concentrations. Of course, below the depth of the ^3He maximum (~ 500 m depth), the simple relationship breaks down, but to the extent that the local source of these tracers will be in the upper waters, this is not a concern. The relationship between nitrate and ^3He also changes in the near surface waters (a kind of 'waterfall' effect at low excess ^3He in Fig. 3) due to strong biological consumption of nutrients in the euphotic zone. The most conservative approach is to use the bulk upper thermocline. ^3He -nitrate relation, that is, $0.080 \mu\text{M kg g}^{-1}$ (per mil) $^{-1}$; because the choice of a steeper slope, as may be indicated by the shallower waters, would lead to a greater nitrate flux estimate. With this slope, I then calculate a mean annual nitrate flux of $0.56 \pm 0.16 \text{ M m}^{-2}$ per yr. This flux would support an annual mean new production rate of $\sim 3.7 \text{ M (carbon) m}^{-2}$ per yr. Such an extension, of course, is dependent on knowledge of the C/N ratio in new production.

Thus a third, independent estimate of new production has been obtained one which agrees well with estimates based on oxygen cycling. The attraction of this latest estimate is that it represents an average over two years, and is based on yet another component of the biogeochemical system. It also resolves some concerns about whether the nutrient budgets are adequate to support the proposed productivity levels. Aside from the general issue of biological productivity, however, how does one rationalize the apparent disparity between this and the 'conventional' nitrate flux estimate based on diapycnic processes? An obvious candidate is isopycnic processes, which have been argued to dominate ventilation of the main thermocline⁴. An additional contender is the existence of nutrient injection events associated with mesoscale features, such as reported elsewhere¹⁴. It appears that only a few such events are required annually to support the computed flux. Using a euphotic zone lifetime of 1 day for

nitrate, the probability of observing such a nitrate pulse directly is $\sim 1\%$, so that direct observation is difficult. However, the geochemical constraints imposed by tracers such as ^3He provide us with important quantitative flux estimates unobtainable by any other means.

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A procynosuchid cynodont from central Europe

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Diversified assemblages of non-mammalian synapsids (or mammal-like reptiles) have long been known from the Upper Permian of southern Africa¹ and the Russian Platform². In contrast, Upper Permian deposits in central and western Europe have rarely yielded remains of terrestrial vertebrates. The German Kupferschiefer (basal Zechstein 1) and its English equivalent, the Marl Slate, have yielded several reptiles: the gliding diapsid *Coelurosauravus*³ (also known from the Lower Sakamena Formation of Madagascar^{3,4}), the archosauromorph *Protorosaurus*⁵, and the pareiasaur *Parasaurus*⁶. The Cuttie's Hillock Sandstone of northern Scotland has yielded the pareiasaur *Elginia* and two dicynodont synapsids and is regarded as equivalent to the *Daptocephalus* zone of southern Africa^{7,8}. We report here the discovery of an incomplete left dentary of *Procynosuchus* from a fissure-filling in limestones, the so-called Randkalk, of the lower Zechstein in the Fisseler quarry, 1 km south of Korbach, northern Hessen (West Germany). This cynodont mammal-like reptile has previously only been known from the uppermost Permian of sub-Saharan Africa^{1,9}. Its presence supports a latest Permian (Tatarian) age for the European Zechstein and, together with other tetrapods common to Europe and Africa/Madagascar, indicates a wide distribution of Late Permian terrestrial vertebrates.

The Randkalk (AlCa)¹⁰ was deposited along the margin of the basin during the middle of Zechstein 1 and subsequently underwent subaerial exposure and karst formation during a temporary regression of the sea at the beginning of the upper

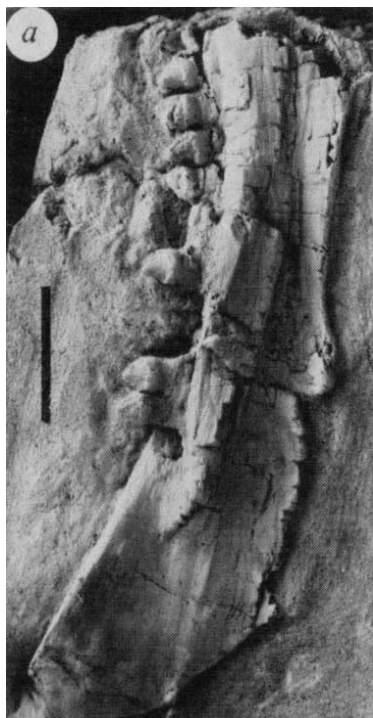


Fig. 1 Left dentary of *Procynosuchus* sp. (PIM-N1225), lower Zechstein, near Korbach, northern Hessen (West Germany). *a*, Lingual view of dentary, (dusted with ammonium chloride; courtesy of V. Krantz). *b*–*d*, Lingual views of tooth crowns of eleventh (*b*), eighth (*c*) and second (*d*) preserved postcanine tooth (slightly restored). Scale bars: *a*, 1 cm; *b*–*d*, 1 mm.

Zechstein 1. The fissure, which is 12 m deep and up to 2.8 m wide, is filled by yellowish to purplish dolomitic mudstone that is capped by the Upper Werra Clay (Tlr) (refs 10, 11), which was deposited by another marine transgression at the end of Zechstein 1. The fissure-filling is locally rich in mostly non-diagnostic bone fragments. The geological context permits precise lithostratigraphic placement of the fossiliferous deposit in the middle of the Zechstein 1.

The specimen, housed in the palaeontological collections of the Institut für Geowissenschaften of Johannes Gutenberg-Universität in Mainz (PIM-N1225), is slightly crushed and exposed in lingual view (Fig. 1*a*). The dentary lacks the symphyseal region and smaller portions of the posterior end. At least part of the coronoid bone is preserved but its sutural outline is indistinct. The dentary has a moderately developed coronoid process. The tooth-bearing ramus is long and low. A shallow longitudinal sulcus, presumably for the dental lamina, extends below the lingual alveolar margin. Due to post-mortem loss of the splenial, the internal groove on the lingual aspect of the dentary is exposed.

The crowns of eight postcanine teeth are preserved, the anteriormost of which is badly damaged. Empty alveoli and gaps indicate the presence of four additional postcanines in the preserved portion of the jaw. A crypt for a replacement tooth is present at the posterior end of the tooth row. Each tooth has a single large cusp that is bounded below and lingually by a cingulum in the region of medial swelling of the crown (Fig. 1*b*–*d*). A corresponding buccal cingulum is absent. The principal cusp of each tooth is slightly recurved, especially on the

more anterior postcanines, and curves lingually. Sharp ridges extend up along the anterior and posterior edges of the crown to the pointed tip. The lingual cingulum connects these ridges. It is weakly developed on the anterior postcanines (Fig. 1*d*), but bears distinct cusps on the more posterior teeth (Fig. 1*b*). The anteriormost and posteriormost cusps, which are situated at the base of the anterior and posterior ridges of the crown, respectively, appear as accessory cusps on the posterior postcanine teeth. Faint ridges and grooves are developed along the lingual aspect of the crowns. Synostosis between the alveolar wall and the root is apparent on the penultimate preserved postcanine.

The Korbach specimen is virtually identical in its structure with dentaries of *Procynosuchus delaharpeae* from the *Daptocephalus* zone of southern Africa¹ and the coeval Madumabisa Mudstones of Zambia⁹. It appears to differ only in the anteroposteriorly concave ventral margin of the tooth-bearing ramus, but this can be accounted for largely by the relative orientation of the jaw in the matrix. In the absence of additional diagnostic material for the German procynosuchid, we refer to PIM-N1225 as *Procynosuchus* sp., while emphasizing its very close similarity to *P. delaharpeae*.

The discovery of *Procynosuchus* from the German Zechstein provides important new evidence for a wide geographical distribution of Late Permian terrestrial vertebrates. This distribution may reflect changes in continental configuration created by the junction of Euramerica and Gondwanaland, as well as large-scale climatic changes during the late Palaeozoic¹². It is in agreement with recent evidence for a decrease in floral provinciality¹³ near the end of the Permian after an earlier period of distinct realms among terrestrial floras.

There has been considerable uncertainty concerning the chronostratigraphic placement of the Zechstein relative to the Lower Permian sequence (Rotliegend of the German stratigraphical nomenclature) of central Europe. As noted, *Coelurosauravus* also occurs in the Lower Sakamena Formation of Madagascar, which has been correlated in its entirety with the Tatarian on the basis of palynological evidence⁴. The presence of *Procynosuchus* in the lower Zechstein supports the correlation of the Zechstein with the upper Tatarian which has been suggested by palaeomagnetic data¹⁴. The Illawarra Reversal, which ended the late Palaeozoic Reversed Interval and occurs in the uppermost Rotliegend of central Europe, is found in the lower Tatarian of the Russian Platform and in the Capitanian (upper Guadalupian) of the southwestern United States. Consequently, the sequence of the Upper Permian in central and western Europe covers a much shorter interval of time than do those of eastern Europe and North America. The sediments of the Zechstein sequence were apparently deposited very rapidly; this is supported by palynological data¹⁵ and varve counts from evaporites¹⁶.

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A new A4 amyloid mRNA contains a domain homologous to serine proteinase inhibitors

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The amyloid proteins isolated from neuritic plaques and the cerebrovasculature of Alzheimer's disease are self-aggregating moieties termed A4 protein¹ and β -protein^{2,3}, respectively. A putative A4 amyloid precursor (herein termed A4₆₉₅) has been characterized by analysis of a human brain complementary DNA⁴. We report here the sequence of a closely related amyloid cDNA, A4₇₅₁, distinguished from A4₆₉₅ by the presence of a 168 base-pair (bp) sequence which adds 57 amino acids to, and removes one residue from, the predicted A4₆₉₅ protein. The peptide predicted from this insert is very similar to the Kunitz family of serine proteinase inhibitors. The two A4-specific messenger RNAs are differentially expressed: in a limited survey, A4₇₅₁ mRNA appears to be ubiquitous, whereas A4₆₉₅ mRNA has a restricted pattern of expression which includes cells from neuronal tissue. These data may have significant implications for understanding amyloid deposition in Alzheimer's disease.

Two full-length A4-specific cDNA clones were isolated from a λ gt10 library and were characterized (Fig. 2). Both cDNA clone sequences were identical to the A4₆₉₅ sequence⁴ except for a 168 bp insert (Fig. 2) resulting in an insertion of 57 amino acids and the removal of one residue of the predicted 695 amino-acid sequence of the A4₆₉₅ protein⁴. This 57 amino-acid sequence was compared to the protein sequence database of the Protein Identification Resource. The search revealed extensive similarity between the insert and the Kunitz family of low relative molecular mass serine proteinase inhibitors (Fig. 1): for the proteins compared in the figure the identity ranges from 33 to 48%. This similarity suggests that the A4₇₅₁ insert could act as a proteinase inhibitor.

To investigate the expression of A4₇₅₁ and A4₆₉₅ mRNAs, two

synthetic oligonucleotides were synthesized. One oligonucleotide corresponds to 60 bases of the 168-bp insert (insert probe) and the other oligonucleotide spans the insert point, possessing 15 bases on either side (junction probe). Under hybridization wash conditions favouring longer oligonucleotides the junction probe is stable when bound to A4₆₉₅ mRNA (30 bp perfect match), but unstable when bound to A4₇₅₁ mRNA (15 bp match). These probes were used to characterize the A4₆₉₅ and A4₇₅₁ cDNA representation in four cDNA libraries prepared from human RNA derived from a SV40-transformed fibroblast cell line, lymphocytes, and normal and Alzheimer's disease brain (see legend to Fig. 2). Insert-positive clones were identified in all libraries; however, only the brain libraries contained junction-positive clones. These results suggested that the expression of the A4-specific transcripts might be differentially regulated. We examined this possibility by characterizing steady-state mRNA levels in several different sources.

Polyadenylated RNA was prepared from human cultured cell lines and from human brain sections. The cultured cells used were HeLa, MRC5 and IMR-32 (see legend to Fig. 3). Tissue samples represented normal cerebellum, frontal and parietal cortex and frontal cortex from an Alzheimer's disease patient. An identical pair of Northern blots were prepared and probed with either the insert or junction oligonucleotide. Both blots were then stripped and reprobed with an actin cDNA probe as an internal control. The results of the Northern analysis (Fig. 3) reveal that each oligonucleotide specifically detects a 3.2–3.4 kilobase (kb) mRNA, consistent with the size of the mRNA encoding the putative A4 amyloid precursor^{4–7}. The junction probe, however, hybridizes to only a subset of the samples, those of neural origin (Fig. 3a, lanes 1, 6–9). No mRNA is detected in the HeLa or MRC5 samples using the junction probe even in the deliberately overexposed autoradiograph shown. In contrast, the insert probe detects sequences in all samples (Fig. 3b). The relative expression levels of the two mRNAs were also examined by RNA dot blot using total RNA prepared from several additional cell lines (P.G.-D. and P.P., data not shown). A human glioblastoma line (U-87-MG) and two human neuroblastoma lines (SK-N-MC and SK-N-SH) all contain mRNA species which hybridize to the insert probe, but none of these samples show significant hybridization to the junction probe above background levels (using MRC5 RNA as the negative control).

At least two types of A4-specific mRNA (and presumably protein) exist which are differentially regulated. We have also determined that the inserted sequence is entirely and exclusively encoded on a separate exon (J.M., F.F. & D.H. unpublished observation). From information on Alzheimer's disease amyloid

Protein	290	300	310	320	330	340	% Identity	Ref.
A4 ₇₅₁ insert	EVQSEQAETGPGKARISRWYFDVTEGKQAFYFYGGGGNRRNFDTEEYQMAVGGSAI						–	–
Bovine pancreatic inhibition	DFCLEPPYTGPKARIIRYFYNAKAGLCQTFVYGGCRAKRNNFKSAEDCMRTGGAI						47	30
Bovine serum inhibition	DFCLEPPYTGPKAAMIRYFYNAKAGFCQTFVYGGCRAKSNFKSAEDCMRTGGGA						42	31
Bovine colostrum inhibition	DLQQLPQARGPKAALLRYFYDSTSNACEPFTYGGCGNNDNFETTEMCLRIEPPQ						42	32
Human ITI Domain I	DSQQLGYSAGPOMGMTSRFYNGTSMACETFYGGMGNGNMFVTEKECLQTRTV						40	33
Domain II	AACNLPVIRGPCRAFIQLWAFDAVKGKVLFPYGGCGNGNKFYSEKECKREYCGVP						46	
Bovine ITI Domain I	DSQQLDYSQGPCLGLFKRYFYNGTSMACETFLYGGMGNNLFLSQKECLQTRTV						33	34
Domain II	EACNLPVIRGPCRAFIQLWAFDAVKGKVLFPYGGCGNGNKFYSEKECKREYCGIP						46	

Fig. 1 Comparison of the amino-acid sequences of the A4₇₅₁ insert with Kunitz proteinase inhibitors. The original computer search was conducted against the Protein Identification Resource database using the FASTP routine of Pearson and Lipman²². Only mammalian proteins are compared although homologous proteins which are members of this family have also been isolated from reptiles, molluscs and coelenterates (reviewed in ref. 23). ITI, inter- α trypsin inhibitor. Only the 57 residues that overlap with the A4₇₅₁ insert are displayed. Numbering is based on the A4₇₅₁ predicted protein sequence as defined in Fig. 1. The 13 residues boxed are invariant in the other members of this inhibitor family and are all conserved in the A4₇₅₁ insert. The percentage identity of each sequence with the A4₇₅₁ insert sequence is shown. Human and bovine ITI domain I is glycosylated at Asn-310.

It is premature to conclude that A4₆₉₅ mRNA is expressed only in cells of neuronal origin. In fact, preliminary experiments indicate that HL-60, a promyelocytic leukaemia line, expresses both forms (P.P., unpublished observation). *In situ* hybridization analysis will be necessary to obtain a complete understand-

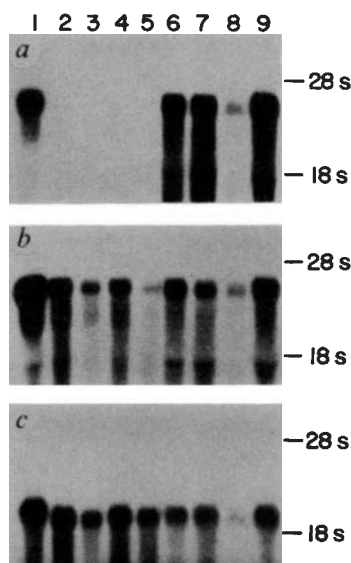


Fig. 3 Northern analysis of A4₆₉₅ and A4₇₅₁ mRNA species. Total or cytoplasmic RNA was isolated from human adult brain sections or cells propagated in culture. The cultured cells are HeLa, an epithelial-like cell derived from a cervical carcinoma; MRC5, a primary fibroblast line from fetal lung tissue; IMR-32, a mixed culture developed from a peripheral neuroblastoma with the predominant cell of neuroblast-like characteristics and the minor type resembling a hyaline fibroblast. All cell lines used are available from the American Type Culture Collection. Total RNA preparations from brains were isolated by the guanidine thiocyanate/LiCl method of Cathala *et al.*²⁴. A cytoplasmic fraction from confluent monolayers of HeLa and MRC5 cells was prepared by lysis of the cells in a hypotonic buffer of 10 mM Tris pH 7.5, 10 mM KCl, 1 mM MgCl₂, 1% Triton-X100, 0.1% sodium deoxycholate for 10 min at 4 °C, homogenization in a Dounce with a 'B' pestle for 10 strokes, and pelleting the nuclei at 2,500g for 5 min. The supernatant was mixed with guanidine thiocyanate, and RNA was pelleted through CsCl by the method of Chirgwin *et al.*²⁵. Total RNA from the same cells was obtained by lysis of the cells directly into guanidine thiocyanate followed by CsCl fractionation as above. RNA (100–400 µg) was fractionated by oligo(dT) cellulose chromatography by the method of Aviv and Leder²⁶. Samples containing poly(A)⁺ RNA were divided into two aliquots, run on duplicate formaldehyde/1% agarose gels, and blot-transferred to nitrocellulose by the method of Thomas²⁷. Nitrocellulose filters were hybridized either to the junction oligonucleotide (5'-CTGCTGTGTAGGAACCTCGAACACCTCTT-3') or the insert oligonucleotide

(5'-CGCCGTAAGAATGGGGCACACTTCCTTCAGTCA
CATCAAAGTACCAGCGGGAGATCA-3')

which had been labelled with a 15–30 nucleotide-long homopolymeric tail using [α -³²P]dCTP and terminal deoxynucleotidyl transferase as previously described²⁸. The buffers used for hybridization contained 10% dextran sulphate, 5 × Denhardt's reagent, 50 mM sodium phosphate (pH 6.7), with SSC and formamide concentrations dependent on the probe used—junction oligonucleotide: 20% formamide, 6 × SSC; insert oligonucleotide: 34% formamide, 4 × SSC; actin: 50% formamide, 4 × SSC. Blots were washed to a final stringency of 1 × SSC at 55 °C, and exposed to Kodak X-AR film for 3 days. Both blots were then stripped and rehybridized to a human β -actin cDNA insert²⁹ which had been labelled by nick-translation with [α -³²P]dCTP²⁰. Re-exposure (6 h) of the filter used in *a* is shown in *c*; re-exposure of filter shown in *b* is very similar and is not presented. The RNA samples displayed are: Lane 1, total IMR-32; 2, total MRC5; 3, total HeLa; 4, cytoplasmic MRC5; 5, cytoplasmic HeLa; 6, normal cerebellum; 7, normal frontal cortex; 8, AD frontal cortex; 9, normal parietal cortex. *a*, RNAs hybridized with the junction oligonucleotide; *b*, RNAs hybridized with the insert oligonucleotide; and *c*, RNAs hybridized with human β -actin cDNA. Ribosomal RNAs used as internal size markers are shown. The normal brain samples were obtained from two different individuals, 98 years of age (frontal cortex and cerebellum samples) and 60 years of age (parietal cortex sample), both lacking clinical signs of dementia. The Alzheimer's disease sample was obtained from a 97-year-old patient displaying overt clinical indications of the disease. The tissue samples were obtained 4, 14 and 5 h post-mortem from the 98, 60 and 97-year-old individuals, respectively. Because different amounts of RNA may be contained in each sample, determination of the relative amounts of each A4-specific species in the different samples is not possible.

ing of the distribution and expression levels of these two A4-specific mRNAs in normal and in pathological conditions such as Alzheimer's disease. The single Alzheimer's disease cortex sample examined here shows no striking differences in the relative amounts of each mRNA compared to the normal cortex samples.

The similarity of the 57-amino-acid insert to a family of proteinase inhibitors suggests that it may have a similar function. The biosynthesis and physiological role of this proteinase inhibitor family are not well understood. Bovine pancreatic trypsin inhibitor is derived from a larger precursor¹⁴ and the other members of the family may be as well. In each case, the functional inhibitor is ~57 amino acids long or a dimer of two such units. The precursor of the human inter- α -trypsin inhibitor may be functionally active¹⁵. Similarly, the entire A4₇₅₁ protein might function as a proteinase inhibitor or the putative inhibitor domain may be proteolytically excised and function independently. The physiological role of other serine proteinase inhibitors is the regulation of a single proteinase¹⁶. The analysis of the physiological function of the A4₇₅₁ insert and the identification of its *in vivo* target must await the production of the pure protein.

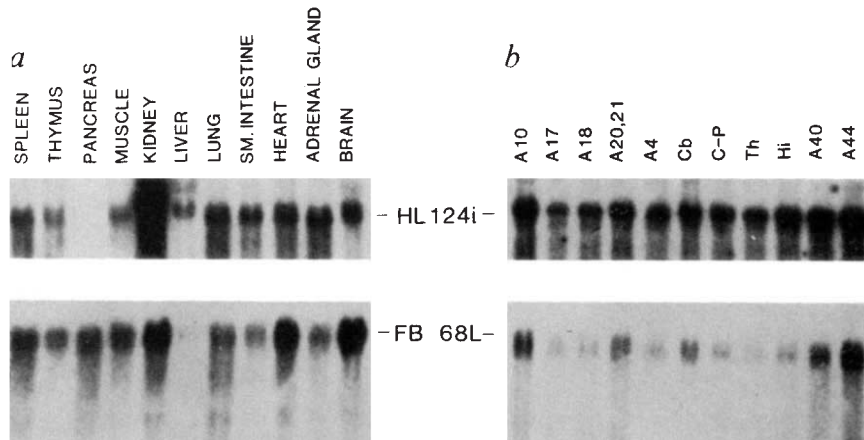
We thank D. Wolf for the SV40-transformed fibroblast library, E. Stoelting for photographic work, C. Boyle and L. Brown for manuscript preparation, C. Bryant and T. Scheuermann for technical assistance and J. Feldman and L. Berg for their efforts in the computer facility. This work was supported by California Biotechnology, Inc. (B.C., P.P., P.G.-D., J.M., J.S., D.H., B.G., F.F.), by the Hartford and McKnight Foundations (I.L.) and the NIH (I.L., K.D., W.W.). The sequence data in this publication have been submitted to the EMBL/GenBank Libraries under the accession number Y00297.

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Fig. 3 Comparison of FB68L- and HL124i-hybridizing mRNAs. Hybridizations of HL124i and FB68L to RNA (20 μ g) from human 20–22-week fetal tissues. Fetal tissue was obtained from mid-trimester elective abortuses under protocols approved by the institutional review board at Brigham and Women's Hospital. The blot to which HL124i was hybridized was equivalent to that used in the FB68L hybridization, except that the former lacked pancreatic RNA; a space has been inserted in the HL124i blot above the pancreatic lane of the FB68L blot. The minor hybridization band above and the APP band, seen in the liver lane, represents non-specific binding of the oligonucleotide probe to 28S ribosomal RNA. *b*, Hybridization of HL124i and FB68L to RNA (10 μ g) from adult human brain subregions: A10, frontal pole of the cortex; A17, striate cortex; A18, extrastriate cortex; A20, 21, temporal association cortex; A4, motor cortex; Cb, cerebellum; C-P, caudate-putamen; Th, thalamus-ventral posterolateral nucleus; Hi, hippocampus; A40, posterior perisylvian cortex-supramarginal gyri; A44, anterior perisylvian cortex-opercular gyri. The two autoradiograms are the result of two independent hybridizations with the same filter. Methods of RNA isolation and hybridization with FB68L have been previously described⁶. Hybridizations with the oligonucleotide were carried out in $5\times$ SSC and 50% formamide at 30 °C, followed by three 30 min. washes in $3\times$, $2\times$ and $1\times$ SSC at room temperature.



does not inhibit trypsin but may interact with other serine proteases¹⁴, shows less similarity (44%) to the predicted HL124i sequence. The nucleotide sequence for domain II (ref. 13) shares 57% homology over 171 bp with HL124i. The polypeptide encoded by HL124i contains many amino acids in consensus positions characteristic of a functional Kunitz domain, including the six cysteine residues that are conserved among Kunitz-type protease inhibitors from cow, snake, turtle and edible snail (N. Ramesh, personal communication)¹⁵.

The cDNAs with and without HL124i should represent different messages generated from the APP gene. We have previously demonstrated⁶ that the fetal brain clone FB68L, which recognizes both forms, hybridizes to 3.4–3.6-kilobase (kb) mRNA species in various human fetal tissues. We have compared this pattern with the hybridization of a 22-base oligonucleotide that should recognize only HL124i-containing mRNAs (Fig. 1). This oligonucleotide also detects 3.4–3.6-kb mRNA species, but with striking differences in tissue distribution. Hybridization with FB68L gives relatively high signal intensity in human fetal brain, heart, and spleen and a barely detectable signal in liver, whereas the HL124i oligonucleotide detects the largest amount of mRNA in kidney and uniform levels among the other tissues, including liver (Fig. 3a). Thus, the relative proportion of these alternative forms of the APP message varies from tissue to tissue, reflecting possible differential regulation of RNA processing or stability. In adult human brain, total APP message detected by FB68L is concentrated in association cortex, specifically in Brodmann areas A10, 44, 20/21, and 40 (ref. 6). But hybridization with the HL124i-specific oligonucleotide shows that the APP transcript containing HL124i is distributed homogeneously among the different brain regions. By subtraction, this difference implies that the APP mRNA lacking the HL124i sequence is selectively expressed in association areas of the brain.

The hybridization of the two probes to mRNA from brains of normal individuals, patients with Down's syndrome (DS), and victims of Alzheimer's disease (AD) is shown in Fig. 4. Relative amounts of mRNA were controlled by hybridization with a cDNA for the microtubule-associated protein, tau. With both APP probes, mRNA from DS fetal brain gives a clear increase in signal intensity over normal. Hybridization with FB68L produces a stronger signal in fetal brain mRNA than in mRNA from adult brain⁶, but the HL124i oligonucleotide detects more mRNA in adult brain regions, suggesting that the

APP mRNA lacking HL124i is selectively expressed to a greater extent in the fetal brain. The total APP mRNA labelled by FB68L and the HL124i-containing component do not differ substantially in the adult cerebellum from patients with AD controls. The ratio of FB68L:HL124i signal, however, is lower in the cerebellum from the AD patient. This difference is more dramatic in the adult frontal cortex. Although the signal produced by FB68L is significantly reduced from AD to normal, the HL124i-containing mRNA seems relatively preserved. The mRNA from affected frontal cortex also shows a substantial loss of tau signal, and displays weaker than normal signal for a variety of neuronal and non-neuronal cDNA probes (R.L.N., unpublished data). Therefore, either the HL124i-containing APP mRNA itself, or the cells expressing it may be selectively spared,

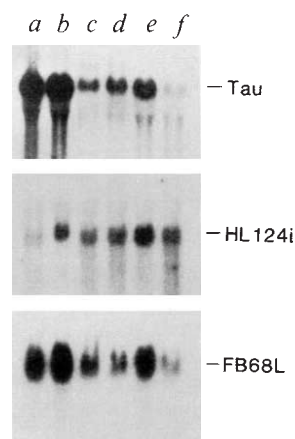


Fig. 4 Northern blot of HL124i and FB68L hybridizations to total RNA (25 μ g) from 19-week normal (lane *a*) and trisomy 21 (lane *b*) brains, adult normal (lane *c*) and AD (lane *d*) cerebellum and adult normal (lane *e*) and AD (lane *f*) frontal cortex. Fetal tissue was obtained from an abortus with a diagnosis of DS and from an age-matched normal abortus. Adult tissue was obtained from autopsy brains of a case of histologically confirmed AD and from an individual without dementing illness. Control hybridization with a cDNA for the microtubule-associated protein tau²⁸ is shown above the results for HL124i and FB68L. The three autoradiograms are the results of independent hybridizations with the same filter.

or even increased, in AD-affected frontal cortex. Conversely, the APP mRNA lacking HL124i or the specific cells containing this species may be severely reduced or eliminated in AD.

It is possible that the potential protease inhibitor domain encoded by HL124i alters the metabolism of APP, perhaps protecting the molecule from degradation by certain serine proteases. Kunitz-type inhibitors are specific for serine proteases such as trypsin, chymotrypsin, elastase, plasmin and cathepsin G (refs 16, 17). Whereas these enzymes could be inhibited by the HL124i domain in APP, other classes of proteases would presumably be refractory to its effects. Thus, the proteolytic intermediates resulting from the metabolism of the APP molecule could differ appreciably, depending on the presence or absence of the HL124i domain. Like human ITI, this domain could have anti-proteolytic activity both in the intact and excised state^{18,19}, giving APP the ability to inhibit the degradation of other proteins. It should be noted that the principal component of the vascular amyloid in the Icelandic form of hereditary cerebral amyloidosis is a variant of cystatin, a cysteine protease inhibitor²⁰. Alpha-1-antichymotrypsin, a serine protease inhibitor distinct from the Kunitz family, is a component of amyloid plaques in AD (C. Abraham, D. J. Selkoe and H. Potter, *Cell*, in the press). As the physiological function of Kunitz-type inhibitors is not understood²³, HL124i could possibly provide the amyloid precursor with a new function unrelated to protease inhibition.

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Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity

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Alzheimer's disease¹ is characterized by cerebral deposits of amyloid β -protein (AP) as senile plaque core and vascular amyloid²⁻⁶, and a complementary DNA encoding a precursor of this protein (APP) has been cloned from human brain⁷⁻¹¹. From a cDNA library of a human glioblastoma cell line, we have isolated a cDNA identical to that previously reported, together with a new cDNA which contains a 225-nucleotide insert. The sequence of the 56 amino acids at the N-terminal of the protein deduced from this insert is highly homologous to the basic trypsin inhibitor family¹², and the lysate from COS-1 cells transfected with the longer APP cDNA showed an increased inhibition of trypsin activity. Partial sequencing of the genomic DNA encoding APP showed that the 225 nucleotides are located in two exons. At least three messenger RNA species, apparently transcribed from a single APP gene by alternative splicing, were found in human brain. We suggest that protease inhibition by the longer APP(s) could be related to aberrant APP catabolism.

We first screened various cell lines by RNA-blot hybridization using a synthetic oligonucleotide probe (AM-1, Fig. 1a), and found APP mRNA to be expressed strongly by human glioblastoma (ATCC HTB14, 16, 17), neuroblastoma (ATCC HTB10, 11) and neuroglioma (ATCC HTB148) cell lines, but only weakly

by hepatoma (HepG2), monocytic (THP-1) and lymphoblastoid (RPMI1788) cell lines (data not shown). Expression by glioblastoma ATCC HTB14 was strongest, and this cell line was therefore chosen for the cDNA cloning.

The cDNA was cloned in two separate fragments for efficiency, the *Bam*HI fragment (5'piece) and *Bam*HI-*Hind*III fragment (3'piece), as shown in Fig. 1a. Complete nucleotide sequencing of the *Bam*HI-*Hind*III fragment harboured by one clone showed it to be identical to that in the corresponding part of the reported APP cDNA (ref. 7).

Two species of the *Bam*HI fragment were isolated using AM-3 as hybridization probe, from a library prepared with AM-1 as primer. One was about 1.4 kilobases (kb) long and the other about 1.6 kb. The nucleotide sequence of the shorter cDNA (later ligated to form full-size cDNA pAPP695, coding for the 695-amino-acid peptide APP695) was identical to that of the 1,376-base-pair (bp) *Bam*HI fragment of the reported APP cDNA (ref. 7). Sequencing of the longer cDNA (later ligated to form full-size cDNA pAPP770, coding for the 770-amino-acid peptide APP770) showed that it differed only in the presence of a 225-bp insert between nucleotides (nt) 865 and 866 of pAPP695, as numbered from the first base of the putative translation-initiating codon. The amino-acid sequence deduced from the nucleotide sequence of the longer cDNA thus includes a novel 75-amino-acid insert in the highly acidic region of the putative extracellular domain⁷ of the APP (Fig. 1b), with valine, amino acid 289 of the original peptide, replaced by glutamic acid and leucine at respective ends of the insert (Fig. 1c).

The novel sequence of 76 amino acids was screened against the NBRF data base, when we found that its N-terminal 56-amino-acid sequence showed a strong similarity to the basic trypsin inhibitor family (Kunitz type), see Fig. 2 (ref. 12). The spacing of its six cysteines is consistent with the spacing found in the minikrinle structure common to this family. Extensive congruency with the highly conserved region of this family (underlined in Fig. 2) is also seen, including the presence of a

Table 1 Relative trypsin activity in COS-1 cells transfected with APP cDNAs (mean $\pm$ s.e.m.)

Plasmid	None	pSVMT-APP695	pSVMT-APP770
run 1	100.0 $\pm$ 15.0	94.0 $\pm$ 12.4	29.0 $\pm$ 15.9 [†]
run 2	100.0 $\pm$ 10.4	101.0 $\pm$ 5.5	36.8 $\pm$ 23.5*

The average value of "none" is set as 100%. Lysate of mock-transfected cells intrinsically showed 47–58% inhibition compared with lysate-free control. *Bam*HI (partial)-*Hind*III fragments of pAPP695 and pAPP770 were inserted into a mammalian expression vector between a mouse metallothionein-I promoter and an SV40 polyadenylation sequence. The plasmid (10 μ g) was transfected into 4×10^6 COS-1 cells by electroporation (3 μ F, 400V, 1.0–1.3 ms, twice, with 30 s interval). The transfected cells were cultured in Dulbecco's modified minimum essential medium with 10% fetal calf serum, and the medium changed after 24 h. Cells were collected, 41 h later and homogenized in extraction buffer (0.1M triethanolamine, 0.3M NaCl, 0.01M CaCl₂, pH 7.8). Inhibition of trypsin activity was measured as described^{21,22} with modification. Cell lysate containing 100 μ g protein was incubated with trypsin for 5 min, and the trypsin activity measured using *N*- α -benzoyl-DL-arginine-7-amino-4-methyl-coumarine (Sigma) as a fluorogenic substrate.

* $P < 0.05$, $^{\dagger} P < 0.01$, Student *t*-test.

basic amino acid (denoted by an asterisk in Fig. 2) in the active site^{13,14}.

In Southern blot analysis of *Bam*HI-digested lymphocyte DNA from one normal individual and eight Alzheimer's disease patients with a 212-bp *Taq*I(nt 862)–*Ava*I(nt 1,073) fragment of pAPP770 (Fig. 1c) as hybridization probe, only a 6.6-kb band was commonly observed, suggesting that the 225-bp insert exists as a single copy, and that there is no significant difference among these genomic DNAs (data not shown). Genomic DNA clones containing the region of the 225-bp insert were isolated from the human leucocyte DNA library (Clontech, USA) using the 212-bp *Taq*I–*Ava*I fragment as probe. In the genomic DNA, the 225-bp sequence was located in a 168-bp exon and a 57-bp exon, separated by an intron of ~ 3 kb, with both exons flanked by intron-exon consensus sequences¹⁵. The 168-bp exon (tentatively designated I, among exons H, I, J and K of this region, as defined in Fig. 3a) corresponds to nt 866 to 1,033 of pAPP770, and the 57-bp exon (J) to nt 1,034 to 1,090. Exon I encodes the highly conserved region of the protease inhibitor family.

In vivo expression of the corresponding mRNA species was investigated by RNA-blot hybridization using 24-mer probes AM-11, AM-12, AM-13 and AM-14, complementary to the H-K, H-I, I-K and J-K junction sequences respectively (Fig. 3a). The results show that mRNA species with the H-I and the H-K sequences were both substantially expressed in fetal brain, whereas the mRNA with the H-I sequence was predominant in adult hippocampus. Comparison of mRNA species having the I-K and the J-K sequences shows that both are expressed in similar amounts in fetal brain, but in adult hippocampus the I-K sequence was expressed more than the J-K (Fig. 3b). These results indicate that at least three species of mRNA are expressed *in vivo*, apparently as a result of alternative splicing. In poly(A)⁺ RNA from HTB14 cell, the mRNA species with the I-K and J-K sequences predominated over that with the H-K sequence.

Greatly increased inhibition of trypsin activity was observed with APP770. Plasmids pSVMT-APP695 and pSVMT-APP770, expressing full-length APP695 and APP770, respectively, under the control of mouse metallothionein-I promoter, were constructed and transfected into COS-1 cells (legend to Table 1). The lysate of the cells transfected with pSVMT-APP770 inhibited trypsin activity against a synthetic substrate more strongly than the lysate of either mock-transfected cells or cells transfected with pSVMT-APP695 (Table 1).

It has recently been shown that the APP gene is not duplicated in Alzheimer's disease (both familial and sporadic) (refs. 16–18),

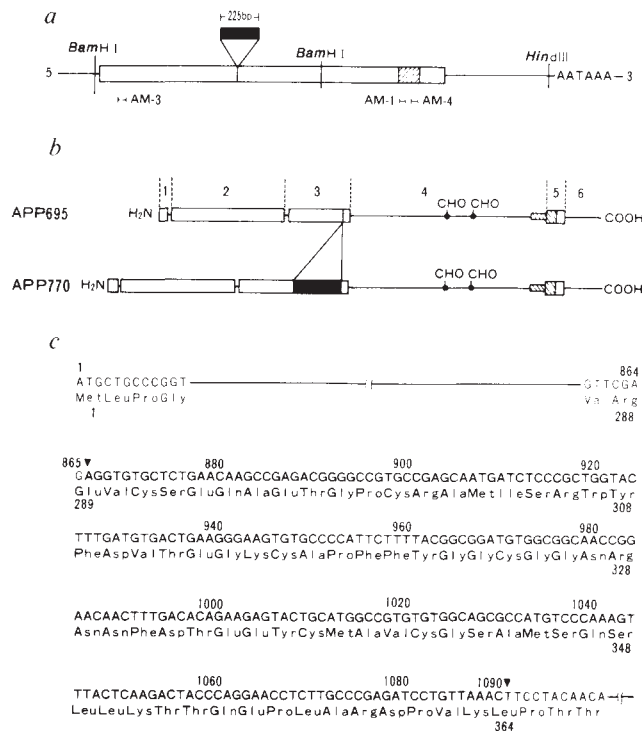


Fig. 1 APP cDNAs (pAPP695 and pAPP770), their predicted proteins, and the nucleotide sequence and deduced amino-acid sequence of the novel insert in pAPP770. *a*, Schematic representation of APP cDNAs. Putative coding region of APP cDNA (ref. 7) in open box; a novel 225-bp insert of pAPP770 in closed box; region coding for peptide of amyloid deposit in hatched box. Positions of oligonucleotide probes AM-3, 4 and primer AM-1 are indicated. *b*, Proposed domain structure of APP695 (ref. 7) and APP770. Domains: 1, signal sequence; 2, cysteine-rich region; 3, highly-negatively-charged region, with novel insert (closed box) in APP770; 4, N-glycosylation-site region; 5, transmembrane segment; 6, cytoplasmic domain. Hatched box, amyloid deposited in the brain. *c*, Nucleotide sequence and predicted amino-acid sequence of the novel insert of pAPP770. Nucleotide residues are numbered as described in the text. The novel insert (gothic) begins at nt 866 and ends at nt 1,090 (between arrowheads). The deduced amino acid sequence is numbered beginning with the amino-terminal methionine. The amino acid residues absent in APP695 are numbers 289–364 (gothic).

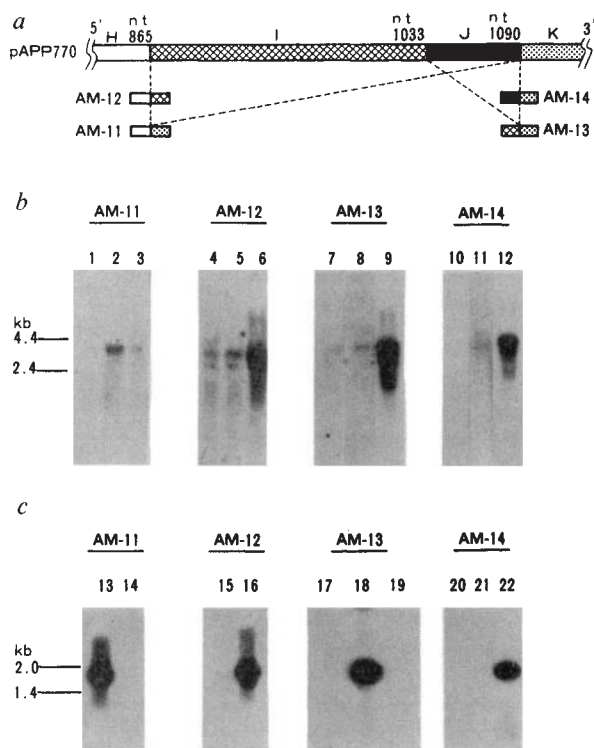
Methods. Glioblastoma HTB14 was purchased from American Type Culture Collection (ATCC) and cultured as recommended by ATCC. Total RNA was extracted²³ from $\sim 5 \times 10^8$ cells. Poly(A)⁺ RNA was isolated by oligo(dT)-cellulose chromatography. (1) Cloning of 3' piece of APP cDNA. Double-stranded cDNA was prepared by oligo(dT) priming using 10 μ g poly(A)⁺ RNA and digested with *Bam*HI and *Hind*III. Fragments of 1–2 kb were isolated by agarose gel electrophoresis and ligated into *Bam*HI–*Hind*III-digested pUC19, followed by transformation into *Escherichia coli* MC1061. Transformants (3.5×10^4) were screened with oligonucleotide probe AM-4 (5'-ACCATGAGTCCAATGATTGCAC-3'). 200 positive clones were obtained, of which 18 were subjected to restriction-site analysis with identical results. The sequence of one clone was determined by the dideoxy method^{24,25}. (2) Cloning of 5' piece of APP cDNA. Double-stranded cDNA was synthesized using oligonucleotide primer AM-1 (5'-CTTCATATCCTGAGTCATGTCG-3') and digested with *Bam*HI. Fragments of 0.7–2 kb were isolated and ligated into *Bam*HI-digested pUC19. Transformants (9×10^3) were screened with AM-3 probe (5'-CATTTCATGTGCATGTTCACTCTG-3') and 20 positive clones were obtained. One of 12 arbitrarily chosen clones had a 1.4-kb insert, as predicted from the reported sequence⁷. The other 11 clones had 1.6-kb inserts. The nucleotide sequence of the 1.4-kb insert and of the 1.6-kb insert was determined. Full-size APP cDNAs, pAPP695 and pAPP770, were constructed by ligation of the 1.4-kb and the 1.6-kb *Bam*HI fragments respectively to the 3' *Bam*HI–*Hind*III fragment.

Fig. 2 Alignment of the N-terminal 56 amino acids of the deduced novel 76-amino-acid insert sequence in APP770 with the known basic trypsin inhibitor family. The residues homologous to the highly conserved sequence of this family are underlined. The basic amino acid residue (Arg) in the active site is indicated by an asterisk. Residues identical to APP770 are boxed. To the left are given the residue numbers of the first aligned amino acid. Proteins: BPTI, bovine pancreatic trypsin inhibitor precursor; BIATI, bovine inter- α -trypsin inhibitor; HIATI, human inter- α -trypsin inhibitor; SA5II, sea anemone proteinase inhibitor 5II (ref. 13); VBPII, Russell's viper venom basic protease inhibitor II

(Protein)	(Number)	290	300	310	320	330	340
APP770	289	EVCSEQAETGPCRAM	I	SRWYFDVTEGKCA	FFYGGCGGNRNN	FDTEEYCM	AVCGSA
BPTI	17	DFCLEPPYTGPCKAR	I	IRYFYNAKAGLCQ	TFVYGGCR	AKRNNFKSA	EDCMRTCGGA
BIATI	59	EACNLPVIRGPCRAF	I	QLWAFDAVKGK	CVRFSGYGGCK	GNNGKFYSQ	KECKEYICGIP
HIATI	80	AACNLPVIRGPCRAF	I	QLWAFDAVKGK	CVLFPYGGCQ	GNNGKFYS	EKECREYCGVP
SA5II	3	GDCELPKVVGPCRAR	F	PRYYNNSSKR	CEKFIYGGCG	GNNNFHTL	ECEKVCVGR
VBPII	5	TFCNLAPESGRCR	GH	LRRYYNLES	NKCKVFFYGG	CGGNANNF	ETRDECRETCGGK

Fig. 3 Expression of APP mRNA in human brain. *a*, Schematic representation of the synthetic oligonucleotide probes (24-mers) designed to detect alternatively spliced APP mRNA species. Regions H, I, J and K correspond to the exons tentatively designated by the same letters (see text). AM-11 (5'-CTGTTGTAG-GAAGTGAACACCT-3') is complementary to the H-K junction; AM-12 (5'-CAGAGCACACCTCTCGAACCACCT-3') to the H-I junction; AM-13 (5'-CTGTTGTAGGAATGGCGCTGC-CAC-3') to the I-K junction; AM-14 (5'-CTGTTGTAGGA-AGTTTAACAGGAT-3') to the J-K junction; nt, nucleotide number. *b*, Northern blot analysis of human brain poly(A)⁺ RNA. Lanes 1, 4, 7 and 10: poly(A)⁺ RNA from adult brain (70-year-old male, hippocampus); lanes 2, 5, 8 and 11: from fetal brain (10-week-old aborted fetus); lanes 3, 6, 9 and 12: from glioblastoma HTB14. Hybridization probes are indicated. *c*, Southern hybridization to determine the specificity of probes. Lanes contain *Bam*HI-digested pSVMT-APP695 (lanes 13, 15, 17 and 20), pSVMT-APP751 (lanes 18 and 21) or pSVMT-APP770 (lanes 14, 16, 19 and 22). Hybridization probes are indicated.

Methods. Total cellular RNAs were prepared from fetal brain and HTB14 cells by the guanidium/CsCl method²³ and poly(A)⁺ RNAs were isolated by oligo(dT)-cellulose chromatography. Poly(A)⁺ RNA from adult brain was purchased from Clontech Inc. Glyoxal-denatured poly(A)⁺ RNA (2.5 µg per lane) was fractionated on 1% agarose gel, run in 10mM sodium phosphate (pH7.0) (ref. 26) and subsequently transferred to Zeta Probe (Bio-Rad). An RNA ladder (BRL) was used as size marker. For Southern blot analysis, pSVMT-APP751 containing the H-I-K sequence was constructed from pSVMT-APP770 using AM-13 as deleter²⁷. *Bam*HI-digested pSVMT-APP695, pSVMT-APP751 or pSVMT-APP770 were transferred to Zeta Probe. Each filter was hybridized with ³²P-labelled oligonucleotide probe in 5×SSC, 25mM sodium phosphate (pH 7.0), 5×Denhardt's solution, 1% glycine, 0.1% SDS at 55°C for 2 h. The filters were washed at 55°C in 6×SSC, 0.1% SDS.



and that the APP gene is not tightly linked to the familial Alzheimer's disease (FAD) locus^{19,20}. Possibly then, the FAD gene product could affect alternative splicing of the APP gene. Although no serine protease(s) which would be inhibited by APP770 or its additional fragments has yet been identified, we suggest that protease inhibition might be involved in the aberrant processing of APP, resulting in the deposit of amyloid β -protein.

The new species of APP cDNA could therefore be important in the investigation of the pathogenesis of Alzheimer's disease and Down's syndrome. During the preparation of this manuscript we learned that two other groups have isolated cDNA clones encoding APP with a sequence homologous to the trypsin inhibitor.

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Membrane currents that govern smooth muscle contraction in a ctenophore

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Ctenophores are transparent marine organisms that swim by means of beating cilia; they are the simplest animals with individual muscle fibres. Predatory species, such as *Beroë ovata*, have particularly well-developed muscles and are capable of an elaborate feeding response¹. When *Beroë* contacts its prey, the mouth opens, the body shortens, the pharynx expands, the prey is engulfed and the lips then close tightly. How this sequence, which lasts 1 s, is accomplished is unclear. The muscles concerned are structurally uniform^{2,3} and are innervated at each end by a neuronal nerve net⁴ with no centre for coordination. Isolated muscle cells studied under voltage-clamp provide a solution to this puzzle. We find that different groups of muscle cells have different time-dependent membrane currents. Because muscle contraction depends upon calcium entry during each action potential, these different currents produce different patterns of contraction. We conclude that in a simple animal such as a ctenophore, a sophisticated set of membrane conductances can compensate for the absence of an elaborate system of effectors.

The feeding response of *Beroë* is triggered by the contact of the prey with the sensory receptors of the lips, and consists of a sudden increase in pharynx volume accomplished by both radial and longitudinal muscles (Fig. 1a, b). Increased tension in the radial fibres stiffens the animal so that contraction of the longitudinals, which shortens the body and opens the mouth, causes water and prey to flow inwards.

Different muscles in the body wall do not have highly specialized individual structures. They are morphologically identical single smooth muscle cells with no T-system, no striation pattern and no coupling between the sarcoplasmic reticulum and the cell membrane^{2,3}. The fibres are 20–40 µm in diameter and up to 6 cm long. They are embedded in an unyielding mesoglea but short lengths may be isolated enzymatically and generate action potentials like those recorded *in situ*⁵. Single action potentials cause appreciable shortening in longitudinal fibres, but not in radials. Upon repeated stimulation both fibre types become progressively shorter.

Action potentials elicited from isolated fibres differ according to fibre type. Those from longitudinal fibres had a marked inflection on their falling phase (Fig. 1c) but this was absent in fibres with branching ends, recognizable as radials (Fig. 1d). As Fig. 1 shows, only the former were prolonged by exposure to the potassium channel blocker, 4-aminopyridine⁶. Spike trains elicited by prolonged depolarization were also different (Fig. 1e); those in radial fibres were maintained, whereas those in longitudinal fibres terminated within a few seconds.

Although there were differences in mean resting potential in the two kinds of fibre (longitudinal fibres -60 s.e.m. 1.4 mV, $n = 25$; radial fibres -66 s.e.m. 1.4 mV, $n = 32$), this was not the reason for the differently shaped action potentials. Figure 2 shows typical membrane currents from identified muscle cells during a 45 mV depolarizing pulse under voltage-clamp; the holding potential each time was -60 mV. Record a, from a longitudinal fibre, is dominated by an early outward current which declined within about 30 ms and was followed by a long-lasting inward current. *In situ* action potentials arise from depolarizing synaptic events simulated in voltage-clamp experiments by a conditioning step imposed before the test pulse. Figure 3a shows two superimposed traces; in one, the membrane

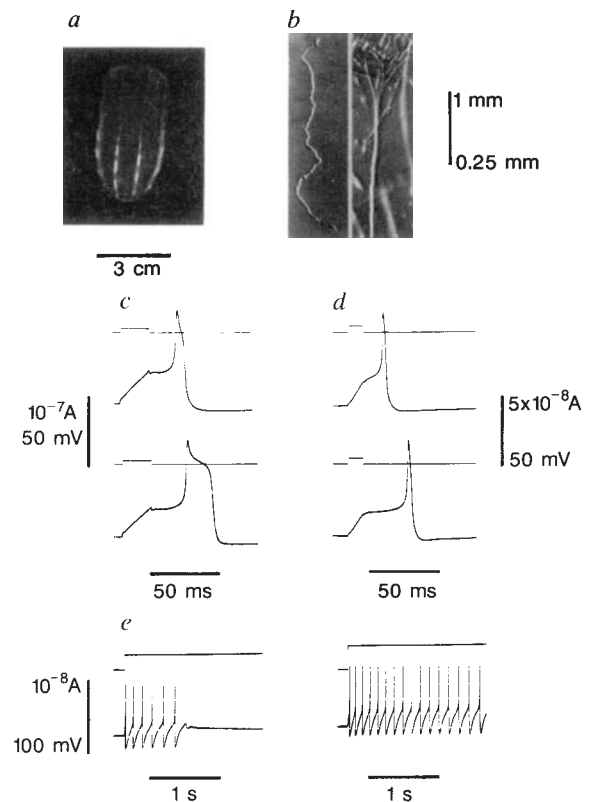


Fig. 1 Electrophysiology of *Beroë* muscle cells. *a*, Freely swimming *Beroë ovata*. The mouth (top) leads to a flattened pharynx often seen as a whitish region through the transparent body wall. The body wall is largely mesoglea through which run endodermal channels and smooth muscle fibres. Radial fibres cross the body wall and are anchored to epithelia on either side; longitudinal fibres are anchored at each end in the same epithelium and may run the whole length of the animal. Circular fibres are also present. *b*, Individual muscle fibres; radial fibres (right; 0.25 mm scale) are identified by their branching ends; (left; 1 mm scale) longitudinal muscle cell fragment isolated by enzymatic digestion (see below). *c*, Intracellular action potentials elicited from longitudinal fibre fragment by a depolarizing current pulse. Top, control record; bottom, effect of 2 mM 4-aminopyridine. The current trace shows 0 mV. *d*, Intracellular action potentials elicited from radial fibre fragment by a depolarizing current pulse. Top, control record; bottom, effect of 2 mM 4-aminopyridine. The current trace shows 0 mV. *e*, Train of action potentials elicited in radial (right) and longitudinal fibres by a maintained depolarizing current. Resting potential of radial fibre -58 mV; longitudinal fibre -48 mV. **Methods.** Specimens of *B. ovata* were collected at Station Zoologique, Université Pierre et Marie Curie, Villefranche-sur-Mer, France during spring 1982, 1986 and 1987. They were kept in sea water at 15°C before use, but all experiments were at room temperature (20 – 23°C). Slices of body wall (2 – 4 cm long) were incubated for 30 min at 30°C in calcium-free saline containing 0.02% trypsin (Sigma, type III) and 0.1% hyaluronidase (Sigma, type III). They were then transferred to cold calcium-free saline and gently shaken until the muscle cell fragments were released. The isolated cells were subsequently bathed in artificial seawater containing (mmol l^{-1}): NaCl, 500 ; KCl, 10 ; CaCl_2 , 11 (or 2); MgCl_2 , 58 ; Tris/Tris-HCl, 5 , pH 7.8 .

was shifted from the holding potential (-60 mV) to -70 mV shortly before the test pulse, the other had an equal period of depolarization to -40 mV. The conditioning depolarization depressed the transient outward current so that the inward current became more visible.

Much of the inward current was blocked by cobalt chloride (10 mM), as if it were carried by calcium⁷. As in vertebrate smooth muscle⁸, a delayed outward current does develop in longitudinal fibres, but only with steps to near 0 mV (data not

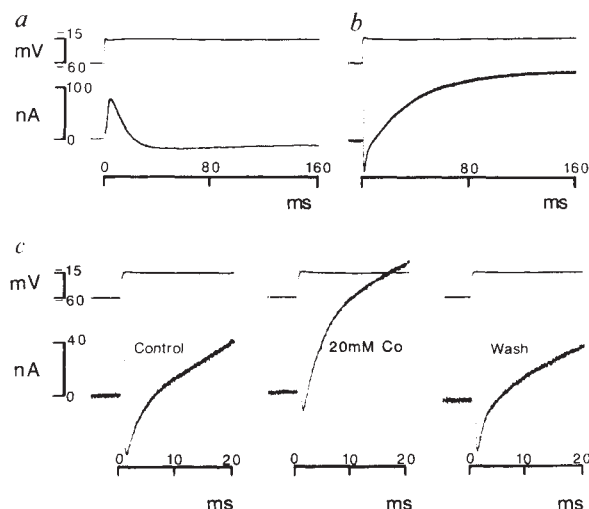


Fig. 2 Ion currents in isolated *Beroe* muscle cells under voltage-clamp. Currents recorded from longitudinal (*a*) and radial (*b*) muscle fibre fragments during a 45 mV depolarizing step (top trace). Holding potential in each case -60 mV. *c*, Effect of 20 mM cobalt chloride on membrane currents recorded from radial fibre fragment. The voltage trace (top) shows the membrane potential change from a holding potential of -60 mV to -15 mV. Lower traces are current records in control conditions and in artificial seawater containing 20 mM cobalt chloride. Net outward current rises due to reduction by cobalt of the prolonged inward current.

Methods. Isolated muscle fibre fragments 0.4–0.6 mm in length were studied with a conventional two-electrode voltage-clamp using KCl-filled glass micropipettes with resistances of ~ 6 M Ω . Calculations from equations derived for a short cable¹⁶ show that current injected midway along a resting fibre (length constant 2 mm) produces a voltage difference between the centre and each end of $\sim 1\%$. Use of a third recording micropipette allowed us to confirm experimentally that even in our most elongated fibres the errors were within 1% for pulses of up to 50 mV. Fibres were in a relaxed state when penetrated, but they invariably shortened during the course of the experiment. The longest experiments lasted for 30 min or more, and in many of these the contractile apparatus appeared to become detached from the cell membrane. When this occurred the cells formed a sphere. The currents recorded from these spheres were qualitatively similar to those recorded from more elongated cells and we used them to test the effect of larger amplitude pulses.

shown). The delayed current often reached a peak within 100 ms, but in some cells the timecourse was slower. Unlike the early outward current, it was abolished by the potassium channel blocker tetraethylammonium chloride (TEA; 50 mM) in the bathing medium. It was cobalt-sensitive, and its current-voltage relationship had an N-shape, attributable to the calcium-dependent potassium conductance⁹ we have demonstrated in *Beroe* by iontophoretic injections of calcium (data not shown).

In isolated longitudinal fibres the action potential peaks at $+20$ mV (s.e.m. 1 mV, $n=18$) and lasts 4–18 ms (at 20% peak amplitude). On this timescale the dominant outward current is the early transient in Fig. 2*a*. At the action potential peak, inward and transient outward currents balance each other. The inward current inactivates only slowly and repolarization starts with an increase in outward current. Once repolarization begins the early outward current declines rapidly and leaves the persistent inward current as a well-defined shoulder. The action of 4-aminopyridine (Fig. 1) and TEA (data not shown) in prolonging the spike, suggests that the final repolarization involves both the remaining early transient (sensitive to 4-aminopyridine, see below) and the slowly developing calcium-activated potassium current (sensitive to TEA).

Although both action potentials peak at the same amplitude, radial fibres repolarize within 2 ms, which is much sooner than

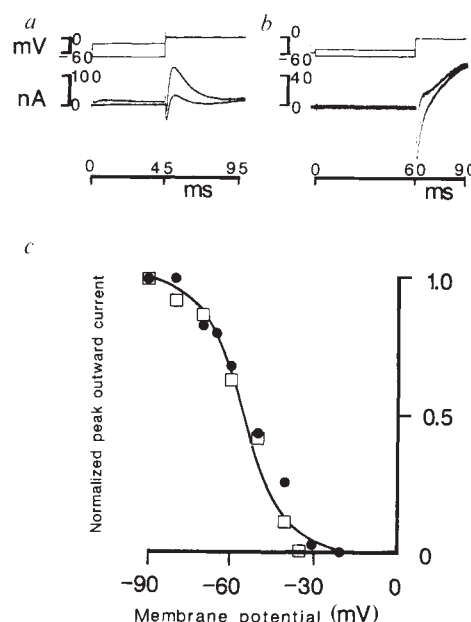


Fig. 3 Transient outward currents elicited by a step depolarization to 0 mV in isolated longitudinal (*a*) and radial (*b*) muscle cells. The voltage steps are shown at the top of each record with the membrane currents below. The timescale is at the bottom. Each set of records consists of two superimposed traces; in one the membrane potential was shifted from the holding potential (-60 mV) to -70 mV shortly before the test pulse. In the other there was an equivalent period of depolarization. *a*, Longitudinal fibre; the effect of a 45 ms conditioning depolarization to -40 mV was to significantly depress the transient outward current. *b*, Radial fibre; the transient seen on the rising phase of the outward current was greatly reduced by a 60 ms depolarizing pulse to -50 mV; the hyperpolarizing pulse to -70 mV increased the outward transient without significantly altering the maintained outward current. *c*, Inactivation of the transient outward current at different membrane potentials. The peak outward current recorded from a typical longitudinal fibre was measured and normalized after 50 ms conditioning steps in the range -20 to -90 mV ($\bullet$). Holding potential, -50 mV; 40 mV test pulse. For comparison, normalized values for the transient outward current from a radial fibre are shown for conditioning pulses over the same voltage range ($\square$). Conditioning pulse duration, 60 ms; holding potential, -60 mV; 55 mV test pulse.

the longitudinals. The reason for this is that radial fibres have an outward current that is both rapidly activated and maintained (Fig. 2*b*). This current was blocked by TEA but, unlike the inward current, showed no sensitivity to external cobalt (Fig. 2*c*).

The small transient on the rising phase of the maintained outward current seen in radial fibres (Fig. 3*b*) resembles the early current recorded from the longitudinals. It too was inactivated by a conditioning depolarization. In both fibre types inactivation occurred even at the resting potential (-60 mV), and was only fully removed by holding the cell at -90 mV (Fig. 3*c*). Both transients were relatively insensitive to 50 mM TEA (data not shown), but were nonetheless carried by potassium; reduced external potassium (from 10 to 5.5 mM) shifted the reversal potential of the current 'tail' seen upon repolarization by an amount predicted by the Nernst equation (from -72 to -85 mV). Transient currents were selectively blocked by 4-aminopyridine (Fig. 4) and were like those found in a variety of both invertebrate^{10,11} and vertebrate^{12,13} tissues.

Although many of the membrane currents in *Beroe* fibres correspond to those in vertebrate smooth muscle^{8,14} the molecular basis of contractility is unknown, and the two forms cannot be assumed to be related. Nevertheless, calcium release from intracellular stores is not required in either, and contractions are abolished by calcium-free solution. In *Beroe*, action

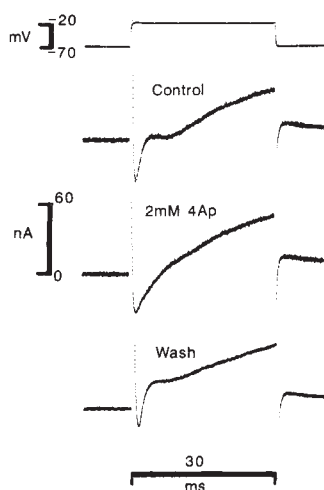


Fig. 4 Effect of 4-aminopyridine on the outward transient in a radial fibre. Membrane currents in response to a 50 mV depolarization (shown at the top of the record) from a holding potential of -70 mV before and after exposure to 2 mM 4-aminopyridine for 10 min. The 4-aminopyridine reversibly blocked the transient on the rising phase of the outwardly directed current.

potentials spread excitation and spike trains generate graded contractions through graded calcium entry. Although radial fibres do not respond mechanically to single spikes, calcium entry during the longer longitudinal action potentials is sufficient to cause shortening. Trains of these prolonged spikes terminate within seconds and ensure a short lived contraction, but the faster radial action potentials support extended spike trains that may maintain the rigidity of the hydrostatic skeleton.

Instead of having groups of structurally different muscle fibres or elaborate patterns of innervation as in higher animals, *Beroe* achieves coordinated movement from muscles whose mechanical response to stimulation depends on the ionic conductances present in the cell membrane. Another version of this strategy appears in the jellyfish *Aglantha*, where slow and fast swimming arise from different regenerative impulses in the same motor axon¹⁵. In both *Beroe* and *Aglantha* coordinated body movements arise from different regenerative impulses in structures of identical morphology. Simple effector systems are driven to produce complex behaviour by complex membrane events.

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Growth-controlling molluscan neurons produce the precursor of an insulin-related peptide

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Insulin and related peptides are key hormonal integrators of growth and metabolism in vertebrates^{1–3}. There is little biochemical evidence for insulin-related peptides in invertebrates, apart from insects for which definitive structural information on these peptides (prothoracicotrophic hormone, PTTH) has recently been obtained⁴. We report here the first complete complementary DNA-derived primary structure of a preproinsulin-related protein from identified neurons in an invertebrate, the mollusc *Lymnaea stagnalis*. We have demonstrated by *in situ* hybridization that transcription of the gene for this molluscan insulin-related peptide (MIP) occurs in the cerebral light-green cells, giant neuroendocrine cells involved in the control of growth, as well as in a pair of neuroendocrine cells called the canopy cells. The insulin-related peptide precursor has the same overall structure as its vertebrate counterparts. The discovery of insulin-related peptides in invertebrates substantiates the evidence for a widespread and early evolutionary origin of the insulin superfamily.

We used a differential hybridization technique to isolate cerebral light-green cell (LGC) cDNAs from a λ gt10 central nervous system (CNS) specific library of *L. stagnalis*⁵. The LGC cDNA clones were obtained by screening replica filters of 20,000 clones with a positive cDNA probe synthesized from messenger RNA of the LGC, and with negative cDNA probes of other parts of the cerebral ganglia and of the digestive gland. The LGC specificity of the clones was tested by *in situ* hybridization using histological sections of the CNS. The nucleotide sequence data of the longest clone (489 base pairs) revealed a single open-reading frame encoding a protein with characteristics of preproinsulins and related proteins (Fig. 1). We therefore called the predicted protein preproMIP. The cDNA clone comprises the complete coding region of preproMIP and 95 nucleotides of a 5' non-translated leader sequence as well as a very short 3' non-translated region. As Northern blotting⁷ indicated that preproMIP mRNA consists of ~ 700 nucleotides (Fig. 2), we infer that most of the 5' non-translated region has been cloned, taking into account both the trailer region and the poly (A) tail of the mRNA.

The alignment of preproMIP and vertebrate preproinsulins unequivocally demonstrates the presence of an invertebrate preproinsulin type of protein in *Lymnaea*. Thus, A and B chains, together with a C-peptide equivalent and a putative signal sequence, are present (Figs 1 and 3). Further comparison shows that overall there is not much similarity in the amino-acid sequence, but that those elements important in the tertiary structure of the molecule have been conserved (Fig. 3). In the MIP A and B chains, cysteines are present at positions typical for the insulin superfamily^{8,9}, suggesting that the two characteristic insulin interchain disulphide bridges in the MIP molecule have been conserved. The presence of two extra cysteines in the MIP A and B chains (at A4 and B-6) indicates that a third interchain disulphide bridge could be present in MIP. The important hydrophobic core residues are either identical with those of other insulins, or are replaced with other equally hydrophobic residues. Thus, MIP may form a tertiary structure, with disulphide bridges, sharp turns and a hydrophobic core, very similar to the known insulins⁹. Interestingly, the MIP A and B

5'-AGUCGUGUACAACUGAGUUUGAACUACUUGCCAGUGUUAUAGAAACGCGGACUARC	59
ACAUAUCAGAUAAUUAUUUUAUAGCUAAAAUCCAAA	110
Met Ala Gly Val Arg AUG GCC GGC GUU CGC	
signal peptide →	
10 20	
Leu Val Phe Thr Lys Ala Phe Met Val Thr Val Leu Leu Thr Leu CUC GUU UUC ACC AAA GCC UUC AUG GUC ACA GUG CUU CUC ACC CUG	155
30	
Leu Leu Asn Ile Gly Val Lys Pro Ala Glu Gly Gln Phe Ser Ala UUG CUG AAC AUU GGC GUG AAA CCU GCA GAG GGU CAA UUU UCA GCU	200
B chain →	
40 50	
Cys Asn Ile Asn Asp Arg Pro His Arg Arg Gly Val Cys Gly Ser UGC AAC AUC AAC GAC CGA CCA CAU CGC CGU GGG GUG UGU GGC UCG	245
60	
Ala Leu Ala Asp Leu Val Asp Phe Ala Cys Ser Ser Ser Asn Gln GCU CUG GCC GAC CUC GUG GAC UUC GCG UGU UCG AGC AGC AAC CAA	290
70 80	
Pro Ala Met Val Lys Arg Asn Ala Glu Thr Asp Leu Asp Asp Pro CCG GCA AUG GUC AAG AGG AAU GCA GAA ACA GAU CUG GAC GAC CCA	335
C peptide →	
90	
Leu Arg Asn Ile Lys Leu Ser Ser Glu Ser Ala Leu Thr Tyr Leu CUG GCA AAC AUA AAG CUG AGC AGC GAG AGC GCU UUG ACC UAC CUG	380
100 110	
Thr Lys Arg Gln Gly Thr Thr Asn Ile Val Cys Glu Cys Cys Met ACC AAG AGA CAA GGG ACA ACC AAC AUA GUG UGU GAA UGC UGC AUG	425
A chain →	
120	
Lys Pro Cys Thr Leu Ser Glu Leu Arg Gln Tyr Cys Pro end AAA CCG UGC ACA CUG AGU GAG CUG AGA CAG UAC UGC CCG UAA UG	469
GAGUUUGACGAUAAAAAA-3'	499

Fig. 1 Nucleotide sequence of preproMIP mRNA, isolated from the LGC of *L. stagnalis*, and its derived amino-acid sequence. The putative proteolytic processing sites (Lys-Arg) are boxed, and the various peptide domains are indicated: namely, the signal peptide, A and B chains and C-peptide. The preproMIP sequence was determined by sequencing⁶ both strands of a 489-base-pair cDNA insert from the longest cDNA of a group of LGC specific clones. The number of nucleotides is indicated at the end of each line. The predicted amino-acid sequence of preproMIP is numbered designating the first methionine as position 1. We estimate that initiation of translation will take place at the Met residue on position 1, although it cannot be excluded that translation initiation occurs at the Met residue on position 13. The end of translation can be assigned to the stop codon at position 124. The MIP precursor has a relative molecular mass (M_r) of 13.4K. The MIP A and B chain together have M_r 6.4K.

chains share additional amino acids with IGF 1 and 2 (Fig. 3). We suppose that the typical monomeric interactions of the insulins do not occur in MIP, because of an important divergence in the carboxy-terminal part of the B chain. Similarly, hexamer formation is not probable, because of divergence at positions B5 and B10⁸.

The signal peptide has a hydrophobic character and probably consists of 31 residues, with Gly at position 31 as the most likely cleavage site¹⁰. Parts of it are homologous with signal peptides of vertebrate insulins¹¹⁻¹⁴. The MIP C-peptide shows no sequence homology with C-peptides of other insulins; this is not unexpected in view of the strong divergence among C-peptides of proinsulins and related peptides of vertebrates⁹.

By applying the *in situ* hybridization technique on histological sections of the CNS with a radioactive preproMIP probe, we found that the LGC are the main cell type to express the MIP gene (Fig. 4). These findings are in agreement with previous experiments demonstrating that the LGC and the median lip nerves (the neurohaemal area of the LGC) contain and release immunoreactive insulin¹⁵. There is also endocrinological evidence that the LGC regulate various processes related to growth in *Limnaea*, such as growth of the soft body parts and the shell, glycogen metabolism and ornithine decarboxylase activity¹⁶⁻¹⁸. The finding that MIP occurs in the LGC, together with the probable function of these cells in growth, fits with current ideas on insulins in insects and vertebrates¹⁻⁴. A further interesting result of the *in situ* hybridization experiments is that both canopy cells appear to express the MIP gene as well (Fig. 4). These neurons are located in the lateral lobes, small ganglia attached to the cerebral ganglia^{19,20}. There is some endocrinological evidence that the lobes are involved in the control of the synthetic and release activities of the LGC^{21,22} but the relationship between the LGC and canopy cells is now more obvious. We believe that the canopy cells are specialized LGC, transmitting stimuli to the LGC groups.

The MIP gene of *Limnaea* may be a member of a small

multigene family, of which the members are expressed in a tissue-specific fashion, as previous studies have shown that a peptide with insulin-like activities is also present in the digestive system^{18,23}. The available evidence suggests that the insulin-related substance of the digestive system has functions different from MIP, and that it is involved in the control of glucose metabolism²³. Thus, in *Limnaea*, there are clear indications that insulin-related peptides are produced by the brain and the

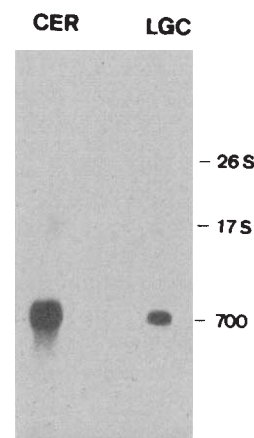


Fig. 2 Size determination of preproMIP mRNA. ~2 µg RNA from the LGC and 10 µg RNA from the cerebral ganglia were glyoxylated, fractionated on a 1.6% agarose gel, transferred to a nitrocellulose filter and hybridized with primer-extended ³²P-labelled preproMIP cDNA. After washing (up to 0.5 × SSC; SSC: 0.15 M NaCl and 0.015 M Na-citrate), the RNA blot was autoradiographed at -70 °C using an intensifying screen. Lane CER, RNA from cerebral ganglia; lane LGC, RNA from LGC. Glyoxylated yeast (*S. carlsbergensis*) ribosomal RNA, 26S (3,400 bases), and 17S (1,800 bases), was used as a size marker⁷.

A Chains		-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
Insulin																												
Lymnaea		Gln	Gly	Thr	Thr	Asn	Ile	Val	Cys	Glu	Cys	Cys	Met	Lys	Pro	Cys	Thr	Leu	Ser	Glu	Leu	Arg	Gln	Tyr	Cys	Pro		
Bombyx		-	-	-	-	Gly	Ile	Val	Asp	Glu	Cys	Cys	Leu	Arg	Pro	Cys	Ser	Val	Asp	Val	Leu	Leu	Ser	Tyr	Cys	-		
Hagfish		-	-	-	-	Gly	Ile	Val	Glu	Gln	Cys	Cys	His	Lys	Arg	Cys	Ser	Ile	Tyr	Asn	Leu	Gln	Asn	Tyr	Cys	Asn		
Guinea pig		-	-	-	-	Gly	Ile	Val	Asp	Gln	Cys	Cys	Thr	Gly	Thr	Cys	Thr	Arg	His	Gln	Leu	Gln	Ser	Tyr	Cys	Asn		
Rat 1		-	-	-	-	Gly	Ile	Val	Asp	Gln	Cys	Cys	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn		
Human		-	-	-	-	Gly	Ile	Val	Glu	Gln	Cys	Cys	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn		
Insulin-like growth factors																												
IGF 1		-	-	-	-	Gly	Ile	Val	Asp	Glu	Cys	Cys	Phe	Arg	Ser	Cys	Asp	Leu	Arg	Arg	Leu	Glu	Met	Tyr	Cys	Ala.....		
IGF 2		-	-	-	-	Gly	Ile	Val	Glu	Glu	Cys	Cys	Phe	Arg	Ser	Cys	Asp	Leu	Ala	Leu	Leu	Glu	Thr	Tyr	Cys	Ala.....		
Relaxin																												
Porcine		-	-	-	-	Arg	Met	Thr	Leu	Ser	Glu	Lys	Cys	Cys	Glu	Val	Gly	Cys	Ile	Arg	Lys	Asp	Ile	Ala	Arg	Leu	Cys	-

B Chains		-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Insulin																																											
Lymnaea		Gln	Phe	Ser	Ala	Cys	Asn	Ile	Asn	Asp	Arg	Pro	His	Arg	Arg	Gly	Val	Cys	Gly	Ser	Ala	Leu	Ala	Asp	Leu	Val	Asp	Phe	Ala	Cys	Ser	Ser	Ser	Asn	Gln	Pro	Ala	Met	Val	-	-	-	
Bombyx		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Hagfish		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Guinea pig		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Rat 1		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Human		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Insulin-like growth factors																																											
IGF 1		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
IGF 2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Relaxin																																											
Porcine		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Fig. 3 Amino-acid comparison of the MIP A and B chains to those of other invertebrate and vertebrate insulin(-related) peptides^{4,8}. The sequence similarity of MIP with insect PTTH and the vertebrate counterparts is 20–40%, whereas that among vertebrate insulins is 55–95%. Compared with the insulins, MIP shares additional amino-acid residues with IGF 1 and 2: namely Glu at A5, Asp at B13 and Phe at B17. In the MIP A and B chains, cysteines are present at positions A6, A7, A11, A20, B7 and B19, an arrangement typical for the insulin superfamily. Two additional cysteines, at positions A4 and B-6, are present in MIP. Like all insulins, MIP has a glycine at B8, but glycines at positions A1, B20 and B23 that are present in most insulins are not found in MIP. In contrast two glycines appear in MIP, at positions A-3 and B5 that are not found in vertebrate insulins. Most residues of the hydrophobic core of the globular structure of insulin are conserved in MIP: namely, A2, A11, A13, A16, A20, B11, B19 but B15 is Val instead of Leu. A3, A19, B6, B12 and B18 residues contribute to the hydrophobic nature of the molecule and are identical or conserved as hydrophobic.

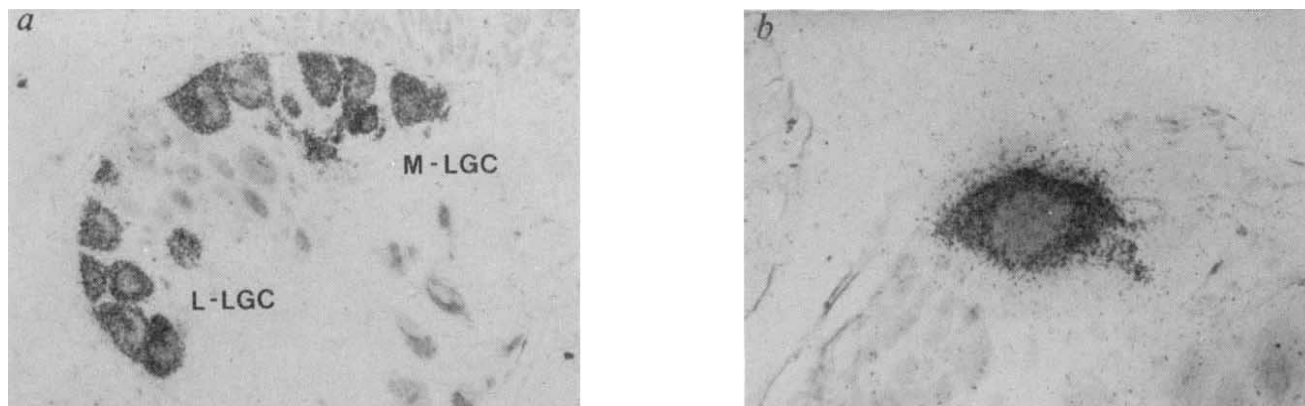


Fig. 4 Localization of preproMIP mRNA in sections of the cerebral ganglia of *L. stagnalis* by *in situ* hybridization using a ³⁵S-labelled preproMIP cDNA. *a*, Hybridization of preproMIP mRNA in the lateral (L-LGC) and median (M-LGC) group of LGC in the right cerebral ganglion. Magnification $\times 120$. *b*, Hybridization of MIP mRNA in the canopy cell of the lateral lobe of the right cerebral ganglion. Magnification $\times 300$.

Methods. Cerebral ganglia were dissected, frozen in freon 22, cooled with liquid nitrogen, and lyophilized at -45°C for 24 h; fixation was performed in paraformaldehyde vapour (1 h, 60°C), followed by embedding in paraplast; 7- μm sections were adhered to glutaraldehyde activated slides, hydrolyzed in 0.2 N HCl for 10 min, rinsed in water, immersed in 1% Triton X100 for 10 min, rinsed in water, fixed in 2% paraformaldehyde in phosphate buffered saline (PBS) for 4 min, rinsed in PBS with 0.2% glycerine, rinsed in PBS, upgraded in 100% ethanol and air-dried, prehybridized in a moist chamber with 50% formamide in a solution containing 50% formamide, $6\times\text{SSC}$, $5\times\text{Denhardt's}$ solution, 2 mM dithiothreitol, $10\mu\text{g ml}^{-1}$ salmon sperm DNA and $200\mu\text{g ml}^{-1}$ transfer RNA for 1 h at 37°C , upgraded in ethanol and air-dried, hybridized for 17 h at 37°C with a ³⁵S-labelled primer-extended (specific activity preproMIP cDNA probe 2×10^8 d.p.m. μg^{-1} ; used at 10 ng per slide without carrier DNA) under the same conditions as for the prehybridization; washed at 60°C in $2\times\text{SSC}$ for 30 min; washed in $0.1\times\text{SSC}$ for 30 min and in $0.1\times\text{SSC}$ for 10 min, upgraded in alcohol, dipped in Kodak NTB 2 emulsion, exposed for 48 h and developed.

digestive system; in vertebrates it is still not known whether insulin is made by neurons in the brain²⁴.

Although MIP has important characteristics in common with various vertebrate insulins, IGF 1 and 2, relaxin and PTTH of the silkworm, it possesses new features not found in any of these peptides. This indicates that MIP is another branch of a relatively ancient superfamily of related hormones and growth factors regulating growth and metabolism.

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Processing of a minimal antigenic peptide alters its interaction with MHC molecules

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Before their recognition by T lymphocytes, protein antigens generally require processing by antigen-presenting cells¹. In a poorly understood series of events, the protein antigen is internalized, transformed and re-expressed on the surface of the antigen-presenting cell in association with gene products of the major histocompatibility complex (MHC). Small peptides derived from the native protein can be recognized in the absence of antigen processing²⁻⁵, suggesting that processing involves proteolytic degradation. These peptides are thought to mimic the naturally produced peptide fragment. We describe here a synthetic peptide antigen of this type which does not require processing but which is nevertheless further processed by splenic antigen-presenting cells. Interestingly, this processing event specifically alters the interaction of the peptide with the class II MHC (Ia) molecule, markedly affecting both its potency as an antigen *in vitro* and its immunogenicity *in vivo* (IR gene control).

The proliferative T-cell response to pigeon and moth cytochrome *c* in B10.A mice is induced by recognition of the antigen in association with the $E^k_\beta:E^k_\alpha$ Ia molecule^{6,7}. The dominant antigenic determinant is in the C-terminus of the cytochrome *c* protein within the sequence 95-103 (refs 8, 9) and the synthetic peptide 93-103(93E) (ELIAYLKQATK in the single-letter amino-acid code) can stimulate antigen-specific T cells in the absence of processing¹⁰. Functional analyses using T-cell clones and synthetic peptides identify residue 99 as part of the epitope, the site that interacts with the T-cell receptor, and residue 103 as part of the agretope, the site that interacts with the Ia molecule. Residue 95 also affects the T-cell response to this peptide, but its role is not clearly defined^{8,9}.

To study the function of residue 95, a peptide analogue was synthesized based on the sequence 93-103 but containing a tyrosine (95Y) in place of the parent isoleucine (95I). This substitution causes a decrease in antigenic potency (Figs 1a and 2a); with B10.A splenic antigen-presenting cells (APC), more 95Y than 95I is necessary to achieve a comparable degree of stimulation of the T-cell clone A.E7. The average decrease in potency resulting from the 95Y substitution is 17-fold $\pm$ 4.9 (geometric mean and standard deviation, $n=16$). The two peptides 95Y and 95I were then assayed with B10.A(5R) splenic APC and the same T-cell clone (Figs 1b and 2b). B10.A(5R) mice express $E^b_\beta:E^k_\alpha$ rather than $E^k_\beta:E^k_\alpha$, and many cytochrome *c*-specific T cells from B10.A mice can recognize antigen in the context of either of these closely related Ia molecules⁶. With B10.A(5R) APC, the tyrosine substitution has the opposite effect: 95Y is significantly more potent than the parent 95I. The average increase in potency resulting from the substitution is 10-fold $\pm$ 4.6 ($n=12$). An effect of the haplotype of the Ia molecule on the apparent antigen specificity of the T-cell response has previously been observed with other cytochrome *c* analogues, and was explained by invoking a direct interaction between the Ia molecule and the antigen¹¹. The fact that 95Y is less potent than 95I with B10.A APC, and is more potent with B10.A(5R) APC for the same T-cell clone, implicates residue 95 as part of the agretope, the site controlling the interaction with the Ia molecule.

A second experiment, however, casts doubt on this simple interpretation. To block processing, B10.A and B10.A(5R) splenocytes were fixed with paraformaldehyde before introduction of the antigen³. Fixed APC cannot induce proliferation or IL-2 production from normal IL-2-producing helper T-cell clones, but they can still stimulate IL-3 production^{12,13}. Fixation by paraformaldehyde has a striking and unexpected effect on the presentation of 95I and 95Y (Fig. 1c and d). Comparison of B10.A and B10.A(5R) APC reveals that the relative potencies of the two peptides are the same; 95Y is less potent than 95I with both APC. When processing is blocked, therefore, the haplotype of the Ia molecule does not affect the response, and evidence for an interaction between residue 95 and the Ia molecule disappears.

The change in the order of potency of 95I and 95Y with B10.A(5R) APC on fixation is caused by the disproportionate decrease in stimulatory capacity of the peptide 95Y (Fig. 1b and d). Although some change in potency of 95I occurs on fixation, the effect on 95Y is much more dramatic. This difference does not result from a direct modification of either the APC or the antigen by the paraformaldehyde, as similar results are obtained when antigen processing is blocked with chloroquine³. In these experiments we used the B-cell hybridoma LB which expresses $E^b_\beta:E^d_\alpha$ and is functionally equivalent to B10.A(5R) APC. The chloroquine treatment does not affect the potency of 95I, but it causes a 10-fold reduction in the potency of 95Y (Fig. 1e and f).

Despite the similar patterns observed when processing is blocked, viable B10.A and B10.A(5R) APC display different patterns of presentation of 95I and 95Y (Fig. 1a versus b, 2a versus b). This raises the possibility that B10.A and B10.A(5R) APC differ in their antigen-processing routes, despite their derivation from H-2 congenic mice. Different nominal antigens could then be generated by the two APC. To investigate this possibility, (B10 $\times$ B10.A) F_1 APC, expressing both $E^k_\beta:E^k_\alpha$ and $E^b_\beta:E^k_\alpha$, were used. When A.E7, a T-cell clone that can recognize either of the Ia molecules, is stimulated, both 95I and 95Y are presented efficiently (Fig. 2c); the pattern is roughly the combination of patterns seen with the parental APC (Fig. 2a and b). The T cell LMB10 (ref. 14), specific for $E^k_\beta:E^k_\alpha$ and unresponsive to $E^b_\beta:E^k_\alpha$, was then assayed. As expected, with B10.A APC, 95Y is less potent than 95I (Fig. 2d), and with B10.A(5R) APC no response is seen (not shown). But with (B10 $\times$ B10.A) F_1 APC, 95Y is much less potent than 95I (Fig. 2e). Therefore, with the

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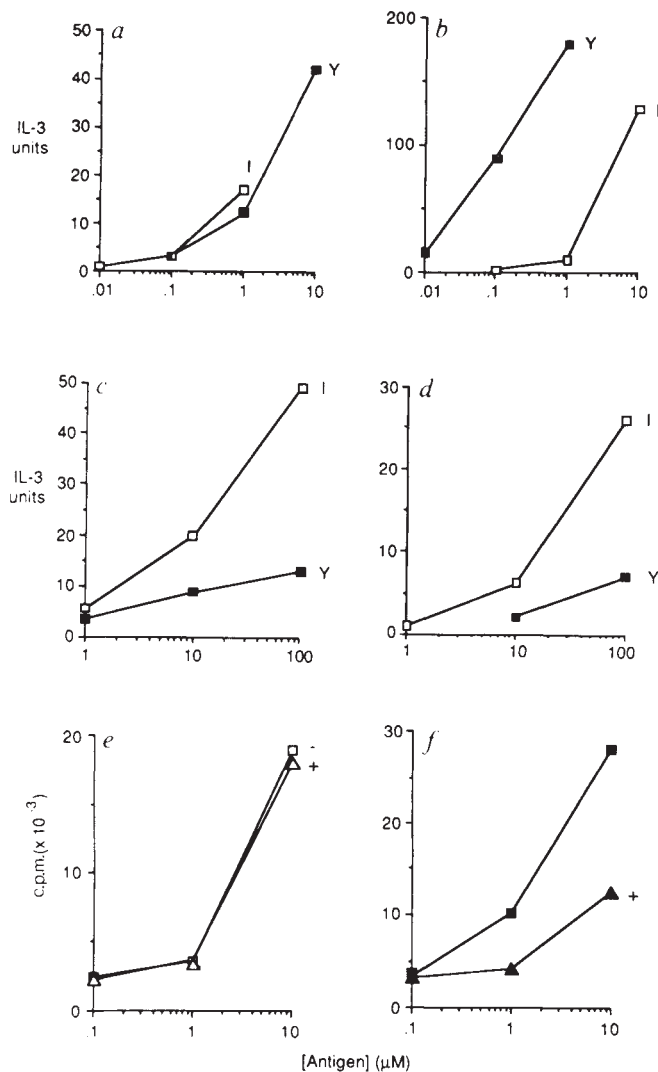


Fig. 1 Antigen processing alters the relative potencies of 95I and 95Y. *a-d*, The T-cell clone A.E7 was assayed with B10.A (*a, c*) or B10.A(5R) (*b, d*) splenic APC and the peptides 95I (open squares) or 95Y (filled squares). *a, b*, Viable APC; *c, d*, APC fixed with paraformaldehyde. Activation was assessed by measuring the production of IL-3 in a secondary assay with the indicator cell line DA-1. *e, f*, B-cell tumour LB incubated in the presence (triangles) or absence (squares) of chloroquine. The T cell A.E7 was then assayed with LB and the peptides 95I (open symbols, *e*) or 95Y (filled symbols, *f*). Activation was assessed after 7 h culture by measuring IL-2 production in a secondary assay with the indicator cell line CTL-L. **Methods.** Peptides were synthesized and purified as previously described⁸. The normal T-cell clone A.E7 (ref. 16) was derived from pigeon cytochrome *c* immunization of B10.A mice. Cells were grown in complete medium containing a 1:1 mixture of RPMI 1640: Eagle's Hanks amino acids with 10% fetal calf serum supplemented with 50 mM 2-mercaptoethanol, 2 mM glutamine and antibiotics. T-cell clones were carried by repeated rounds of stimulation with antigen and irradiated (3,000R) splenocytes followed by rest for 7–21 d. Paraformaldehyde fixation of splenocytes and chloroquine treatment of the B-cell hybridoma LB (ref. 17) were performed as described³. For *a-d*, T cell activation was measured by incubating 2×10^4 T cells with 5×10^5 splenocytes (+/- fixation) and the indicated concentration of antigen in 96-well plates in duplicate culture. After 24 h, supernatants were removed, frozen, serially diluted and assayed for IL-3 activity using the IL-3-dependent cell line DA-1 (ref. 18) ($2-4 \times 10^3$ per well). DA-1 cells were incubated with the supernatants for 24 h, pulsed with [³H]thymidine (1 μCi per well), and after 12–16 h the cells were collected on glass fibre filter strips and the radioactivity incorporated into the DNA determined by liquid scintillation counting. A unit of IL-3 is defined as the reciprocal of the dilution of supernatant required to half-maximally stimulate the DA-1 cells. For *e* and *f*, T-cell activation was assessed by incubating 2×10^4 T cells with 2×10^4 LB cells (+/- chloroquine) and the indicated concentration of antigen in duplicate culture. After 7 h, supernatants were removed and assayed for IL-2 activity using the IL-2-dependent cell line CTL-L (2×10^3 per well). CTL-L assay was identical to that described above for DA-1, except that the results are shown as the proliferation of the CTL-L cells in response to 25% culture supernatant.

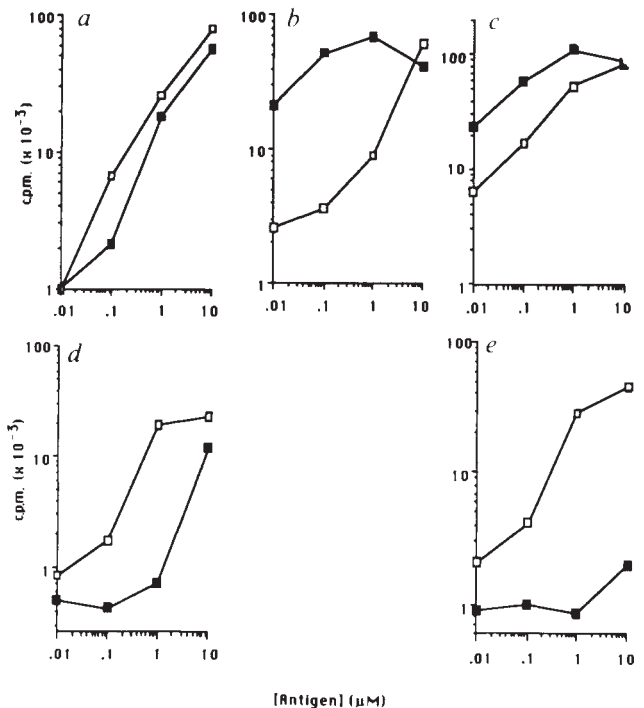


Fig. 2 Change in relative potency of 95I and 95Y with B10.A and B10.A(5R) APC cannot be explained by processing differences. T-cell clones A.E7 (*a-c*) or LMB10 (*d-e*) were assayed with irradiated splenocytes from B10.A (*a, d*), B10.A(5R) (*b*) or (B10 x B10.A)F₁ (*c, e*) mice and 95I (open squares) or 95Y (filled squares). No stimulation of the clone LMB10 was seen with B10.A(5R) APC (10 μM 95I, 553 c.p.m.; 10 μM 95Y, 364 c.p.m.). **Methods.** The T-cell clone LMB10 (clone B10 in ref. 14) was derived and carried like A.E7 (see Fig. 1 legend). T-cell activation was assessed by culturing 2×10^4 T cells with 5×10^5 irradiated (3,000 R) splenocytes with the indicated concentration of antigen in 96-well plates in duplicate culture. After 48 h, proliferation was measured by pulsing the cultures with [³H]thymidine as described in Fig. 1 legend.

same F₁ APC, both patterns of relative potencies of 95I and 95Y are observed (Fig. 2*c* versus *e*), depending only on the Ia molecule involved in antigen presentation to the T cell. It is thus not necessary to invoke a processing difference between B10.A and B10.A(5R) APC. Instead, the results indicate that a processed form of the peptide antigen 95Y, produced in both B10.A and B10.A(5R) APC, is responsible for detecting the polymorphism between $E^k_\beta : E^k_\alpha$ and $E^b_\beta : E^k_\alpha$; the processing event specifically alters the aglycope of this defined peptide. We do not yet know whether residue 95 itself is directly modified, or whether a distal site, influenced by the amino acid at position 95, is modified. Examination of truncated peptides suggests that the N-terminal residues are not removed during processing. The peptide 95–103(95Y) is less potent than 95–103(95I) with both B10.A and B10.A(5R) APC (data not shown), indicating both that the truncated peptides cannot detect the polymorphism between the two Ia molecules, and that the N-terminal residues must be present for the processing event to occur.

To determine whether this processing event is of physiological importance, we studied the *in vivo* immune response. Peptides based on the pigeon cytochrome *c* sequence 93–104 (103, alanine; 104, lysine) show that the presence of alanine at position 103 results in a poor association between antigen and the $E^b_\beta : E^k_\alpha$ molecule¹⁵, causing the pigeon peptide to be poorly presented by B10.A(5R) APC, and to be non-immunogenic in B10.A(5R) mice. The tyrosine substitution at position 95 in the pigeon peptide has a similar effect *in vitro* to that seen in Fig. 1 with the moth peptide: pigeon 95Y is less potent than pigeon 95I with B10.A APC, and more potent with B10.A(5R) APC (data

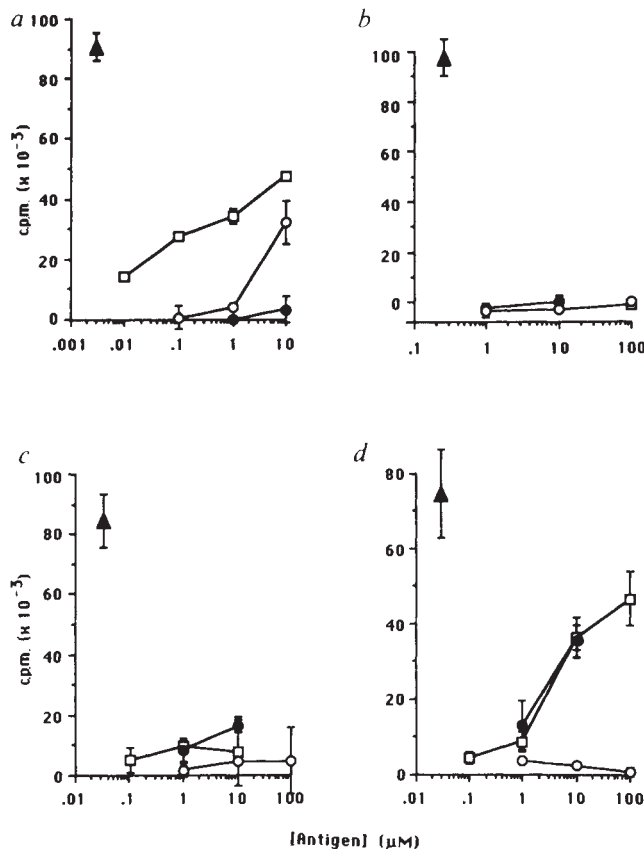


Fig. 3 *In vivo* immunogenicity of pigeon 95I and pigeon 95Y in B10.A and B10.A(5R) mice. B10.A (*a, b*) or B10.A(5R) (*c, d*) mice were immunized with 20 nmol of pigeon 95I (*a, c*) or pigeon 95Y (*b, d*). The proliferation of the lymph node T cells was measured in response to syngeneic APC and pigeon 95I (open circles) or pigeon 95Y (filled circles). Filled triangles, response to purified protein derivative (Connaught Labs). The T cells were also assayed with moth 95I (open squares) to demonstrate that the responding T cells have similar specificity to that expected from immunization with the moth peptides.

Methods. Mice were immunized in all four foot pads and the base of the tail with antigen emulsified in 0.2 ml complete Freund's adjuvant (Difco). After 7 d, the draining lymph nodes were removed and purified by passage over a nylon wool column¹⁹. T cells (4×10^5 per well) were cultured with irradiated (3,000 R) splenocytes (1×10^5 per well) and antigen in flat-bottomed wells for 72 h followed by an overnight pulse with [³H]thymidine, as described in Fig. 1.

not shown). When these peptides are tested *in vivo*, there is a direct correlation between *in vitro* antigenic potency and *in vivo* immunogenicity. The increased *in vitro* potency of pigeon 95Y with B10.A(5R) APC is reflected in an increased *in vivo* immunogenicity in B10.A(5R) mice (Fig. 3*c* versus *d*). Similarly, the decreased *in vitro* potency of the pigeon 95Y peptide with B10.A APC corresponds to a diminished *in vivo* immunogenicity in B10.A mice (Fig. 3*a* versus *b*). Thus, a single amino-acid substitution at position 95 turns B10.A mice into non-responders and B10.A(5R) mice into responders. The processing event that increases the potency of the 95Y peptide with B10.A(5R) APC *in vitro* is therefore also operating *in vivo* and is controlling immune responsiveness.

We demonstrate here that a peptide that does not require processing to be recognized by T cells can nevertheless be processed under physiological conditions, leading to an altered association with MHC gene products, and a corresponding change in specific immune responsiveness *in vivo*. The ability of defined peptides to bind to purified Ia molecules *in vitro* (where processing cannot occur) correlates with the *in vivo*

'choice' of Ia molecules recognized by T cells specific for that peptide¹⁵. But there are several quantitative and qualitative discrepancies in this correlation. The data presented here suggest that some of these discrepancies could be caused by a failure to analyse the true nominal antigens active in the *in vivo* stimulation of T cells.

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Isolation of an HTLV-1-like retrovirus from patients with tropical spastic paraparesis

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Tropical spastic paraparesis (TSP) is a slowly progressive myelopathy associated with increased serum and cerebrospinal fluid antibodies to the human T-lymphotropic retrovirus type I (HTLV-I) (ref. 1), and has been observed in many regions of the world^{2–4}. A similar condition known as HTLV-I-associated myelopathy⁵ occurs in the Kagoshima prefecture of Japan. Recent^{6,7} but controversial^{8–10} reports suggest involvement of virus related to HTLV-I in multiple sclerosis. Magnetic resonance imaging and electrophysiological studies indicate that TSP lesions are like multiple sclerosis¹¹ in that they are disseminated throughout the nervous system. Complete virus from patients with TSP has proved difficult to isolate^{12,13} using techniques successful in adult T-cell leukaemia cases associated with HTLV-I. Here we report the isolation of an HTLV-I-like virus from T-cell lines derived from the peripheral blood and cerebrospinal fluid of TSP patients. The monoclonal antibody OKT3 was used to generate non-transformed T-cell lines that express HTLV-I antigens. Infectious virus was demonstrated by co-cultivation and complete, replicating virions were visualized ultrastructurally.

T-cell lines were established from two HTLV-I seronegative controls and seven TSP patients (Table 1). Purified peripheral blood lymphocytes (PBL) or cerebrospinal fluid (CSF) cells

Table 1 Detection of HTLV-I associated gene products in T-cell lines derived from peripheral blood and cerebrospinal fluid

TSP patient	Nationality	Earliest passage number when cultures are HTLV-I P19 positive*		% Positive HTLV-I P19 antigen at current passage number	
		PBL	CSF	PBL	CSF
I	Haitian	P8	P7	75% (P29)	60% (P7)
II	Colombian	P11	P9	70% (P25)	50% (P23)
III	Colombian	-	-	0% (P10)	0% (P10)
IV	Colombian	P5	P4	15% (P5)	75% (P11)
V	Colombian	P5	P3	30% (P11)	15% (P10)
VI	Colombian	P3	P2	25% (P11)	50% (P9)
VII	Jamaican	P1	P3	30% (P7)	75% (P4)

* 10% positive, determined by indirect immunofluorescence with the anti-HTLV-I P19 antibody as described in the legend to Fig. 1.

were cultured in the presence of interleukin-2 (IL-2) and irradiated allogeneic feeder cells and the monoclonal antibody OKT3, and passaged every 5–7 days. OKT3 was used as a non-selective activator of T cells as it stimulates the antigen-specific T-cell receptor-CD3 complex^{15,16}. Fixed cytocentrifuge preparations of the T-cell lines were assessed by indirect immunofluorescence with monoclonal antibodies specific for HTLV-I p19 and p24 gag proteins. Twelve T-cell lines derived from the PBL and CSF of six of the seven TSP patients contained large amounts of viral antigen detected by immunofluorescence (Fig. 1a, b). The expression of HTLV-I-related antigens in the TSP cell lines varied with passage history (Table 1). T-cell lines derived from PBL of controls and from patient III did not react with these antibodies. Viral antigen was not detected in either fresh PBL or cultured, unstimulated PBL from the TSP patients.

T-cell lines from patient I were studied in detail and HTLV-I-related gag proteins confirmed by immunoprecipitation. Control sera reacted with a number of cellular proteins (Fig. 2a, lanes B and D). But the TSP sera and the monoclonal antibody anti-P24 specifically immunoprecipitated three proteins with relative molecular masses (M_r) ~ 53,000 (53K), 24K and 22K (Fig. 2a, lanes C and E). In a Western blot, the anti-p24 antibody reacted with similar HTLV-I proteins, believed to represent processed intermediates of the gag protein (A. Bodner, personal communication). Similar patterns were observed using radio-labelled, transformed cell lines infected with conventional HTLV-I (Hut 102). Serum from a patient with adult T-cell leukaemia (ATL) immunoprecipitated the 24K and 22K peptides, but showed no reactivity with the 53K protein.

DNA from the PBL T-cell line from patient I was hybridized with a 9-kilobase (kb) HTLV-I probe¹⁷. Bands corresponding to M_r s 2.4kb, 1.6kb and 1.3kb were observed in the *Pst*I digests (Fig. 2b, lane 2). Identical bands were observed when the control Hut-102 cell line, known to contain multiple proviral copies and integration sites, was probed (Fig. 2b, lane 1). These are consistent with the restriction map of HTLV-I (ref. 17). An *Eco*RI digest of the TSP T-cell line DNA (Fig. 2b, lane 3) produced one band which could represent either monoclonal integration, or integration of multiple proviruses plus flanking sequences. The 2.9-kb *Pst*I band (Fig. 2b, lane 2) is consistent with an interpretation of monoclonal integration.

To determine whether infectious virus was present, PBL from an HLA-mismatched normal individual were co-cultured with irradiated (10,000R) PBL T-cell line of TSP patient I. The co-cultured cells had the HLA phenotype of the recipient PBL, and after the first passage 40% of cells contained HTLV-I-related proteins (Fig. 1c). Recipient cells cultured in the absence of the TSP T-cell line did not react with antibodies raised against HTLV-I. The irradiated TSP T-cell line did not grow when cultured alone.

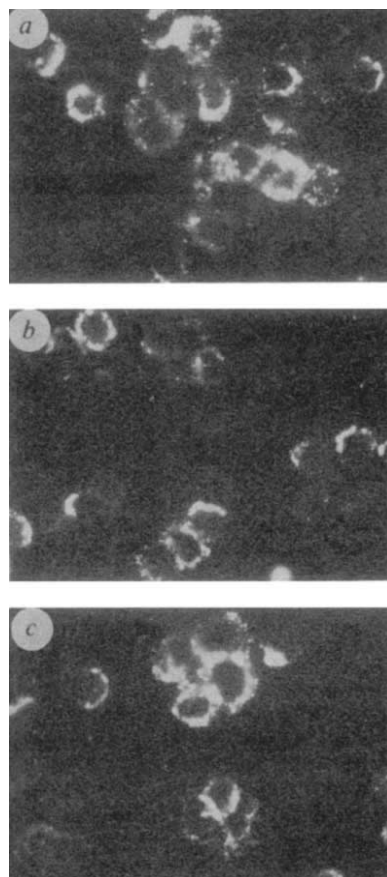


Fig. 1 T-cell lines were derived from PBL by culturing 1.2×10^7 cells in 10 ml RPMI (Gibco, N.Y.) containing 5% human serum (blood group AB) plus 10% IL-2 (Cellular Products, Buffalo) and a 1 in 5,000 dilution of ascites of the murine monoclonal antibody OKT3 (ATCC). Cells were passaged at a density of $5-10 \times 10^5$ per ml; 5×10^5 cells were subcultured, and irradiated (6,000R) allogeneic feeders were added in 5–7-fold excess. CSF T-cell lines were derived by culturing $0.5-1.0 \times 10^5$ CSF lymphocytes in the above medium with irradiated autologous or allogeneic feeders. Indirect immunofluorescence assays were done on acetone-fixed cytospin preparations. Cells were incubated for 30 min with the murine monoclonal antibodies specific for HTLV-I p19 or p24 gag protein (Biotech Research). Slides were washed in phosphate-buffered saline (PBS) and stained for 30 min with an F(ab')₂ sheep anti-mouse IgG conjugated to fluorescein isothiocyanate (FITC; Cappel, PA). Cells were washed in PBS which contained a 1 in 2,000 dilution of 1% Evans Blue to give a red fluorescence under ultraviolet illumination to contrast with the antibody-specific yellow-green FITC staining. a, T-cell lines derived from patient I PBL and stained with the HTLV-I-specific p19 antibody. b, T-cell lines derived from patient II CSF and stained with the HTLV-I-specific p19 antibody. Similar staining was obtained with the anti-p24 antibody. c, Fluorescence with the anti-P19 antibody of cells derived from the co-cultivation of normal PBL with the irradiated (10,000R) T-cell line of the patient I. Similar staining was obtained with the anti-p24 antibody. Co-cultured cells were maintained as described above, except that 0.3% phytohaemagglutinin (GIBCO) was added to the medium.

T-cell lines from patients I, II and IV were used for ultrastructural studies (Fig. 3). Complete and intact viral particles were observed extracellularly in all these lines. Virions were most frequently cell-free (Fig. 3, a–c), but immature virions budding from the plasmalemma were common (Fig. 3, e–g). Mature virions ranged in diameter from 80–120 nm (Fig. 3b, c) and consisted of a nucleocapsid surrounded by an envelope¹⁸. The virions were indistinguishable from those in the HTLV-I carrier

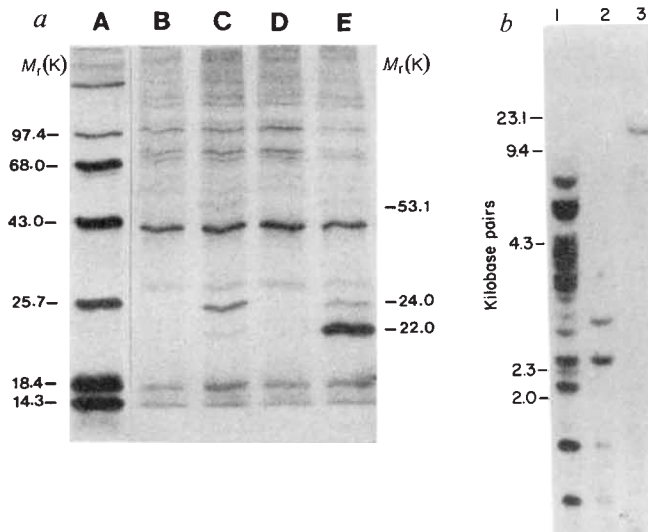


Fig. 2 *a*, The T-cell line (1×10^7 cells) from the PBL of TSP patient I were cultured for one passage in the absence of OKT3 and labelled with 0.75 mCi of [35 S]methionine (Amersham) in 20 ml methionine-free RPMI, which was supplemented with 5% of the normal amount of methionine, for 18 h. After centrifugation and washing with PBS, cells were lysed and immunoprecipitated using a modification of the method of Bellini *et al.*²⁵. Aliquots of lysate containing 3×10^6 d.p.m. were cleared with 100 μ l 10% *Staphylococcus aureus* Cowan I (Sigma) in 300 μ l of radioimmunoprecipitation assay buffer containing 0.5% myoglobin²⁷. The supernatant was incubated for 1 h at 4°C with *S. aureus* which had been *a*, coated first with the IgG fraction of a rabbit anti-mouse IgG (Cappel) and then for 1 h with 10 μ l mouse ascites or *b*, coated directly with the human serum to be tested. The *S. aureus* was washed and the immune complex dissociated and analysed by SDS-PAGE electrophoresis. Lane A, *M_r* standards; lane B, immunoprecipitate with an irrelevant control mouse ascites; lane C, immunoprecipitate with the murine monoclonal anti-p24 (Biotec Research); lane D, immunoprecipitate with an HTLV-I seronegative normal control serum; lane E, immunoprecipitate with the serum of the TSP patient I. *b*, Hut-102 or the PBL T-cell line of patient I was homogenized in 4 M guanidine thiocyanate and the homogenate layered over a cushion of 5.7 M CsCl. After centrifugation at 30,000 r.p.m. for 18 h, the DNA above the cushion was collected and dialysed against 10 mM Tris-HCl pH 7.4, 1 mM Na₂EDTA, and digested with proteinase K (200 μ g ml⁻¹). After extraction with phenol CHCl₃ and dialysis, 10 μ g each DNA was digested with restriction endonucleases and the products separated by electrophoresis through 0.6% agarose and transferred to a Biotrans membrane filter for hybridization to a full-length HTLV-I probe¹⁷ radiolabelled with 32 P in 50% formamide, 0.75 M NaCl at 43°C for 60 h. After washing successively in 2 $\times$ SSC and 0.1 $\times$ SSC in 0.1% sodium dodecylsulphate (SDS) at ambient temperature, and in 0.1 $\times$ SSC in 0.1% SDS at 43°C, the filters were dried and exposed overnight at -100°C to X-ray film with two intensifying screens. Lane 1, Hut-102 DNA digested with *Pst*I; lane 2, TSP T-cell-line DNA digested with *Pst*I; lane 3, TSP T-cell-line DNA digested with *Eco*RI.

cell line, C1-MJ (ref. 19) (Fig. 3*d*). The icosahedral nature of the capsid was evident in some sections, giving hexagonal profile with a honeycomb-like substructure. Viral replication involved the assembly of capsid material beneath the plasmalemma followed by budding (Fig. 3, *e-g*). Smaller circular or tubular profiles in the extracellular space were intermixed among virus particles (Fig. 3*b, c*).

The association of TSP with HTLV-I is based largely on serological findings¹⁻⁵. Demonstration of an HTLV-I-related virus from both PBL and CSF of six TSP patients strengthens the idea that this virus is involved in the pathogenesis of this disorder, but additional criteria²⁰ are required to establish it as the aetiological agent. Infection by HTLV-I may lead to immunopathological or autoimmune abnormalities which cause

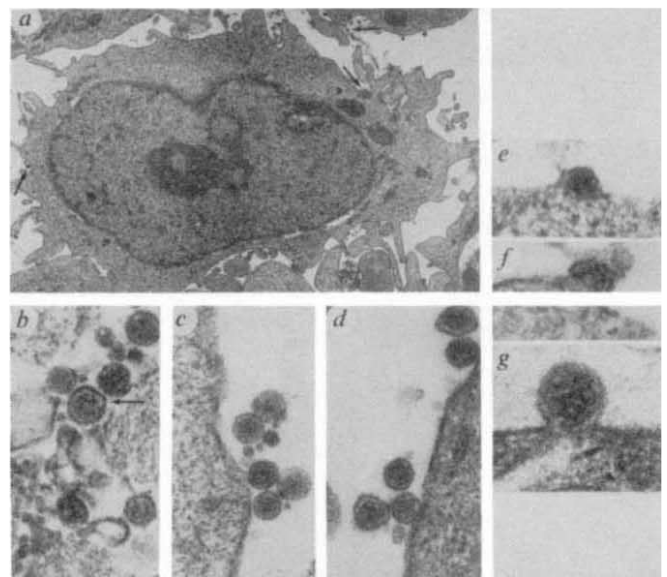


Fig. 3 The T-cell line derived from patient I was used for ultrastructural analysis. A suspension culture containing $\sim 5 \times 10^6$ cells was pelleted at 500g at 4°C, fixed by 2.5% glutaraldehyde in phosphate buffer (pH 7.3) for 60 min, and post-fixed by 1% osmium tetroxide for 60 min. The pellets were then passed through a graded series of ethyl alcohol, cleared in propylene oxide, and embedded in Epon 812. Thin sections for electron microscopy were stained with uranium and lead salts. *a*, Low-power electron micrograph showing the majority of small (10 μ m diameter), densely-staining PBL. Note the homogeneous granular cytoplasm, mitochondria and scattered rough endoplasmic reticulum. The dense particles (arrows) in the extracellular space are virions. Magnification $\times 8,750$. *b*, A small group of virions among smaller tubular profiles which probably represent dissociated capsid material. One virion (arrow) contains a capsid which is doughnut-shaped. Spike material is occasionally seen (upper right) around the envelope. Magnification $\times 100,000$. *c*, A small group of virions along a cell surface. Note that each has a core (nucleoid or capsid) which sometimes has an icosahedral profile, a doughnut shape, or a honeycomb appearance, together with a distinct envelope. Magnification $\times 75,000$. *d*, Group of virions at the surface of a cell from the HTLV-I-infected cell line, C1-MJ (ref. 19). The virions appear identical to those in *c*. Magnification $\times 75,000$. *e-g*, Stages in viral budding. *e*, Accumulation of viral nucleoid beneath a membrane protrusion, magnified $\times 110,000$; *f*, the pouching-out of the viral bud which now contains a horse-shoe shaped nucleoid, magnification $\times 120,000$; *g*, an almost completely budded virion containing a mature nucleoid, magnified $\times 140,000$.

the neurological disease. Elucidation of the mechanism of damage could have broader implications because an association between HTLV-I and multiple sclerosis has been proposed^{7,8}. Neurological abnormalities in TSP are not confined to the spinal cord, and as in classical multiple sclerosis, other sites are involved¹¹. Although three out of six TSP patients carrying an HTLV-I-like virus had magnetic resonance imaging lesions^{14,21} consistent with disseminated involvement, the neuropathological basis for these lesions is not known.

Virus was not demonstrated in the T-cell lines derived from patient III (Table 1) who was the spouse of patient II, and was probably exposed to the same agent. But peripheral blood DNA from patient III gave strongly hybridizing bands with the HTLV-I 9-kb probe. It is not understood why viral antigen was not detected in the lymphocytes of this patient, but there is a precedent in that HIV-1 cannot be isolated from all patients with neurological syndromes associated with AIDS (ref. 22).

A distinct property of conventional HTLV-I is the capacity to immortalize and transform normal lymphocytes^{23,24}. In contrast, the T-cell lines derived from the six TSP patients have remained dependent on IL-2. Further biological differences

between the viruses associated with TSP and conventional HTLV-I have been identified. Co-cultivation with the Hut 102 cell line to infect a CD4⁺ HLA class II-restricted cytotoxic T-cell line, specific for influenza virus, inhibited the cytotoxic function. No inhibition was observed when the influenza T-cell line was infected by the PBL T-cell line of TSP patient I (manuscript in preparation).

The presence of an HTLV-I-related virus in 12 TSP cell lines was demonstrated by T-cell activation with the monoclonal antibody OKT3. Some T-cell lines required the presence of OKT3 as well as IL-2 for growth. Stimulation of the T-cell receptor complex may enhance the endogenous production of virus proteins in the same way as in the activation pathways described for HTLV-I and HIV-1 (refs 25, 26). Collectively, the data indicate that viruses isolated from TSP patients are related to HTLV-I, but may represent variants of HTLV-I, or possibly new members of the human retrovirus family that lack transforming or lytic properties. This would be consistent with the absence of malignancies or lymphoproliferative abnormalities in patients with TSP.

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Conserved organization of the human and murine T-cell receptor β -gene families

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Generation of an immune response depends on the interaction of haematopoietic cell types, among which T cells and their receptors are of central importance. The T-cell receptor is a heterodimer consisting of disulphide-linked α and β -chains, each chain divided into variable (V) and constant (C) regions¹⁻⁴. The β -chain is encoded by the rearrangement of separate variable (V_β), diversity (D_β) and joining (J_β) gene segments during T-cell differentiation^{5,6}. To examine the mechanisms of somatic DNA rearrangement and evolution of the β -gene segments, we have constructed a physical map of the human T-cell receptor β -chain family containing 40 V_β gene segments as well as both C_β gene clusters. A comparison of the published nucleotide sequences of human and murine V_β gene segments reveals 12 examples of gene segments sharing 65% or more interspecies homology. The relative order of these human and murine V_β gene segment homologues is also conserved along the chromosome, apart from more extensive human gene duplication, presumably as a consequence of constraints imposed on evolutionary mechanisms operating to diversify these gene families or of selective pressures operating to maintain order.

Three different techniques were used to construct a physical map of the human β -gene family: (1) deletion analyses of V_β gene segments in T-cell lines⁷; (2) Southern blot analyses of the field-inversion gels⁸ with V_β probes; and (3) restriction enzyme analyses of cosmid clones. The deletion map is constructed as follows. When a V_β gene segment on one human chromosome 7 is rearranged to a previously joined D_β - J_β sequence, usually all the intervening DNA, including V_β gene segments 3' to the rearranging V_β gene segment, is deleted⁹. In a single T cell, if both copies of chromosome 7 undergo V_β to D_β - J_β rearrangements, then the V_β gene segments proximal and distal to the 3' most rearranged V_β gene segment can be determined by their presence or absence, respectively. Hence, a series of these

Table 1 Deletion analysis of V_β gene segments in human T-cell lines

	Germ-line (HeLa)	CEM	MT2	YAM	H9	MAR
$V_\beta 1$	1	0	1	1	1	1
$V_\beta 2$	2	1	1	1	1	1
$V_\beta 3$	4	3	3	3	3	3
$V_\beta 4$	2	1	1	1	1	1
$V_\beta 5$	5	0	0	2	5	5
$V_\beta 6$	6	0	0	0	6	6
$V_\beta 7^*$	3	2	—*	—*	2	3
$V_\beta 8$	5	0	0	2	2	5
$V_\beta 9$	1	1	1	1	1	1
$V_\beta 10$	3	2	2	2	2	2
$V_\beta 11$	2	1	1	1	1	1
$V_\beta 12^\dagger$	7	3	4	6	6	7
$V_\beta 13^\dagger$	6	2	2	4	5	6
$V_\beta 14$	6	5	6	6	6	6

The human T-cell lines presumably undergo V_β rearrangements on both chromosome 7 homologues by using rearrangement mechanisms that lead to a deletion of intervening DNA between the rearranged V_β and D_β gene segments. Numbers represent the number of bands observed by Southern blot analysis in each cell line, using DNA probes specific for each V_β subfamily. No V_β -gene segment deletions were detected in the remaining 14 T-cell lines.

* Not possible to determine because of polymorphisms.

† Three gene segments are shared between $V_\beta 12$ and $V_\beta 13$ subfamilies.

rearranged T-cell lines permits the construction of a deletion map indicating the relative order of V_β gene segments along the chromosome, based on their presence or absence in different T-cell lines.

We have analysed 19 human T-cell lines for V_β gene deletions by Southern blot analysis using V_β probes from each of the 14 V_β subfamilies previously defined¹⁰. V_β gene deletions were detected in five of the human T-cell lines (Table 1). A previous analysis had indicated that germ-line variation in the number of V_β gene segments was infrequent in human populations, and hence the deletions observed could be reliably attributed to β -gene-segment rearrangement¹¹. For example, the $V_\beta 1$ gene segment is deleted in T-cell line CEM but present in MT2, suggesting that it is located between the rearranged V_β gene segments in CEM and MT2. The deletion data divide the V_β gene segments into five groups (Fig. 1a).

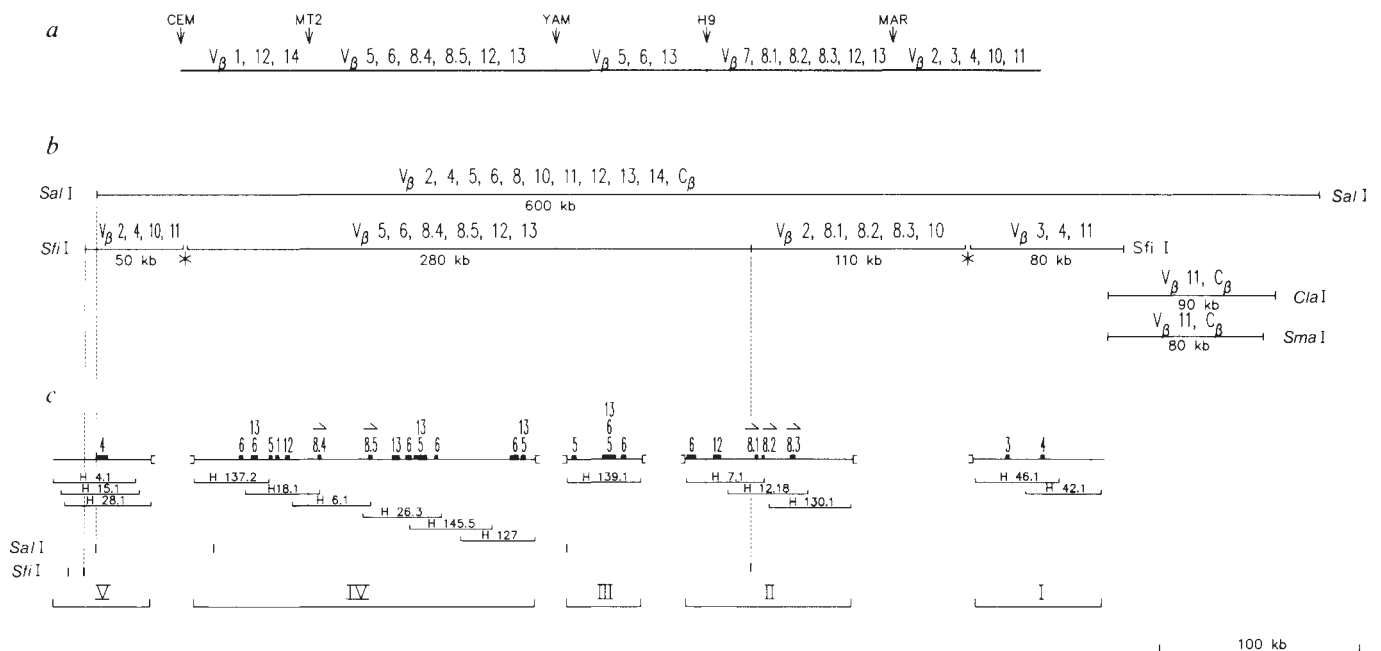


Fig. 1 *a*, The relative order of V β gene segments determined by deletion analysis of T-cell clones. Arrows, location of the C β proximal 3'-most rearranged V β gene segment in each human T-cell line. The V β gene segments located between the deletion end-points in these T-cell lines are indicated. The relative order of the V β gene segments mapped between the T-cell line deletion breakpoints cannot be determined from these analyses. *b*, Field-inversion gel analysis of the V β gene segments. Restriction enzymes used are indicated. Numbers below the horizontal lines indicate the size of each particular DNA fragment in kb. The V β gene segments that have been used to hybridize to the restriction fragments are indicated above the horizontal lines. *c*, Restriction maps of cosmid clones. Cosmid clone H12.18 was isolated from an osteosarcoma U20S cell line. Clones H7.1 and H18.1 were isolated from a human sperm cosmid library and have been described previously¹⁷. The remaining clones were isolated from a HeLa cosmid library. Restriction sites for the SalI and SfiI restriction enzymes are shown. All five members of the V β 8 subfamily have been sequenced¹⁷, and their transcriptional orientations are indicated by horizontal arrows above each member. Dotted lines descending from a position of the 3'-most C β proximal rearranged V β gene segments in the T-cell lines used. Dashed lines descending from b, restriction sites observed in the field-inversion gels. Further attempts to isolate additional clones in this 600-kb region from the three cosmid libraries were unsuccessful, suggesting that all three libraries were deficient in certain regions of the human β -gene family, possibly resulting from cosmid clone instability caused by the presence of highly repetitive DNA sequences.

Methods. See Fig. 2 for methods for field-inversion gel electrophoresis and hybridization conditions. Cosmid libraries constructed from DNA derived from HeLa cells, osteosarcoma U20S cells and human sperm¹⁷ were screened with ³²P-labelled V β DNA probes in routine hybridization and washing conditions, and positive clones purified and mapped by multiple restriction enzyme digestions and partial digestion¹⁷.

Field-inversion electrophoresis of human DNA previously digested with four infrequently cutting restriction enzymes, and Southern blot analysis using 14 V β and C β probes has enabled us to map most of the known human V β gene segments and the two D β J β C β clusters. DNA probes, specific for 10 of 14 V β subfamilies and the C β genes, hybridized to a 600-kilobase (kb) SalI fragment, suggesting that most of the identified V β gene segments and the two D β J β C β clusters are linked on 600 kb of DNA (Figs 1b and 2). When SfiI digests were analysed, V β probes corresponding to 9 of these 10 different subfamilies were found to hybridize to only 4 different fragments (Figs 1b and 2). The order of these SfiI fragments was determined by combining the data from the deletion mapping and the field-inversion gels. For example, the 280-kb SfiI fragment must be located 5' to the 110-kb SfiI fragment because the V β 8.4 and 8.5 gene segments map 5' to the V β 8.1, 8.2 and 8.3 gene segments by deletion, and because one of the members of the V β 2 subfamily hybridizes to the 110-kb fragment but maps by deletion analysis 3' to all members of the V β 8 subfamily. Analyses of the ClaI and SmaI fragments demonstrate that the two D β J β C β clusters are within 80 kb of one member of the V β 11 subfamily, establishing that this V β 11 gene segment is the most 3' of the identified V β gene segments.

DNA probes specific for V β 1, 3, and 7 subfamilies did not hybridize to any SalI fragment, even though these gene segments have been located to the 600-kb SalI fragment by deletion mapping. This may simply reflect the decreased sensitivity of the field-inversion blots compared to normal genomic blots. Indeed, cosmid clone analysis confirms the position of these V β

gene segments as indicated by deletion mapping (see below and Fig. 1c).

We then further characterized the β -gene family with cosmid clones. Twenty cosmid clones were isolated from three independent human cosmid libraries. These clones were completely mapped with restriction enzymes EcoRI, BamHI, HindIII (data not shown), SalI and SfiI. Restriction enzyme analyses indicate that 15 of the cosmid clones can be grouped into 5 clusters (Fig. 1c). The remaining five cosmids overlap with the clones shown, but map differently with respect to the V β gene segments due to restriction fragment length polymorphisms (data not shown). In Fig. 1c, solid boxes above the horizontal line represent restriction fragments that hybridize to DNA probes specific for each of the 14 V β subfamilies. Numbers above the solid boxes indicate which V β subfamily was found to hybridize to these restriction fragments. When more than one number is shown above a single box, this indicates that probes from several V β subfamilies hybridized to the same fragment. Further detailed restriction enzyme analysis indicates that these fragments contain distinct germ-line V β gene segments corresponding to each subfamily. We did not determine the relative order of the V β gene segments contained within these fragments.

The five cosmid clusters were oriented by referring to the field-inversion and deletion maps. For example, cluster II is assigned by virtue of its SfiI site 10 kb 5' to the V β 8.1 gene segment. This site must be the 5'SfiI site on the 110-kb SfiI fragment, because a single copy probe isolated from the 5' side of this site hybridized to an SfiI fragment of 280 kb in length. This indicates that the 280- and 110-kb SfiI fragments are

Fig. 2 Southern blot analyses of V_{β} gene segments on HeLa cell DNA separated by field-inversion gel electrophoresis. Each lane contains 10–15 μ g of DNA. Ligated λ DNA concatemers were used as size standards. Switch times used were 10–60 s for the *Sal*I digest, 3 to 8 s and 3 to 30 s for the *Sfi*I digests, and 3 to 15 s for the *Cla*I and *Sma*I digests.

Methods. High molecular weight DNA for field-inversion gel electrophoresis was prepared as described²³. Field-inversion gel electrophoresis⁸ was carried out at 10 volts cm^{-1} through a 1% agarose (SeaKem, FMC Bio-products) gel in 45 mM Tris, 45 mM borate, 2 mM EDTA buffer, pH 8, for 20 h with a constant 3:1 ratio between the forward and reverse interval. The gel was then stained with 1 μ g ml^{-1} ethidium bromide and exposed to shortwave (254 nm) ultraviolet light for 60 s at an intensity of 2.5 mW cm^{-2} . DNA was transferred to nylon membranes (Zeta-Probe, Biorad Laboratories) in 0.4 N NaOH (ref. 25) for 2 d and neutralized with 0.5 M Tris buffer, pH 8, for 5 min. The filters were hybridized in 50% formamide, $5 \times \text{SSC}$, 0.02 M sodium phosphate (pH 7), 250 μ g ml^{-1} denatured salmon sperm DNA, 0.5% non-fat powdered milk, 1% SDS and 10% dextran sulphate at 37 °C overnight²⁴. DNA probes were purified from 1% low-melting agarose (Seaplaque, FMC Bioproducts) and radiolabelled by random-priming²⁵ to a specific activity of $>1 \times 10^9$ c.p.m. μg^{-1} DNA. Filters were washed three times in $2 \times \text{SSC}$ and 0.2% SDS at 65 °C for 20 min.

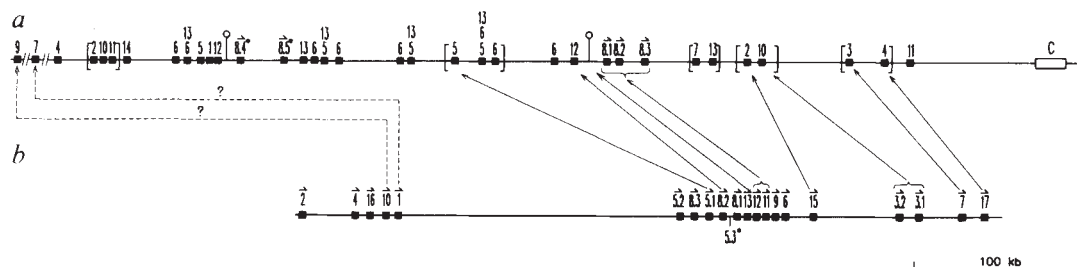
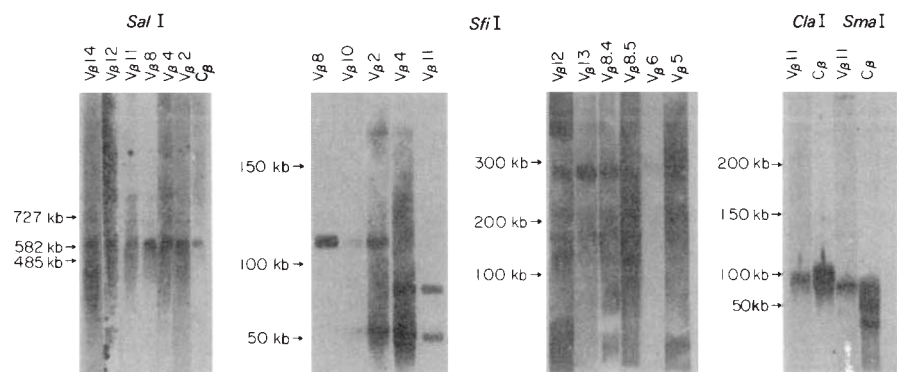


Fig. 3 *a*, Physical map of the human T-cell receptor locus. This figure summarizes the data obtained from deletion analyses of T-cell lines, field-inversion gels and restriction enzyme mapping of the cosmid clones. The relative order of V_{β} gene segments within the brackets could not be determined from these analyses. Moreover, brackets indicate V_{β} gene segments localised by deletional and field-inversion analyses, but not by linked cosmid clones; hence there is an uncertainty of ~10–15 kb in their positions. Horizontal arrows above V_{β} gene segments indicate their transcriptional orientation. The restriction fragments that lack known human V_{β} gene segments but hybridized strongly to a murine $V_{\beta}13$ probe are indicated by open circles. *b*, Physical map of the mouse β -locus^{7,13}. $V_{\beta}17$ represents the V_{β} -gene segment reported by Singer *et al.*²⁶. Asterisk, pseudogenes.

contiguous. We could not determine whether the cosmid clusters I and V and their corresponding 80- and 50-kb *Sfi*I fragments are immediately adjacent to the 110-kb and 280-kb *Sfi*I fragments. It is possible that there are small *Sfi*I fragments not detected by field-inversion gel electrophoresis and which are located between the clusters, as suggested by the presence of a 10-kb *Sfi*I fragment identified by restriction mapping of cosmid clones H4.1, H15.1 and H28.1. As both members of the $V_{\beta}4$ subfamily hybridized to the 600-kb *Sal*I fragment, this indicated that the *Sal*I site in clone H4.1 probably forms the 5' end of this fragment. Moreover, the 600-kb *Sal*I fragment observed in the field inversion gel is likely to a reproducible fragment from partial enzyme digestion because *Sal*I sites were found in clones H137.2 and H139.1.

There are several interesting features of the physical map of the human β -gene family (Fig. 3*a*). (1) All 40 V_{β} gene segments whose positions are identified map to the 5' side of the two $D_{\beta}J_{\beta}C_{\beta}$ clusters. (2) Members of the same subfamily can be adjacent (for example, $V_{\beta}8.1$, 8.2 and 8.3) or highly interspersed (for example, $V_{\beta}4$, 5, 6 and 11). Interspersion of V-gene segments has also been found in the mouse β -gene^{7,12,13} and human immunoglobulin loci^{14,15}. (3) The two $D_{\beta}J_{\beta}C_{\beta}$ clusters are within 80 kb of one $V_{\beta}11$ gene segment, the 3'-most V_{β} gene segment yet identified. (4) The five $V_{\beta}8$ gene segments are known to have a transcriptional orientation in the same direction as the $D_{\beta}J_{\beta}C_{\beta}$ clusters.

Previous analyses by hybridization of interspecies V_{β} -gene segment homology indicated that mouse V_{β} gene segments did not cross-hybridize with human DNA, suggested to be caused

by rapid evolution of V_{β} gene segments¹⁶. This contention is not supported, however, by mutation rate calculations based on germline V_{β} -gene segment sequences¹⁷. Furthermore, comparisons between human and mouse V_{β} -gene segment nucleotide sequences show that 12 out of 17 mouse V_{β} subfamilies have human homologues that are 65–83% identical to these mouse counterparts, whereas a comparison of the V_{β} gene segments between subfamilies in the human or mouse shows similarities of $48 \pm 8.5\%$ (average $\pm$ s.d.) and $47 \pm 6.7\%$ respectively. In virtually all cases, the similarity of mouse–human homologues is significantly (≥ 2 s.d.) greater than that found between members of different human or mouse subfamilies. To confirm this observation and determine whether it could be used to identify additional human V_{β} gene segments, we screened our human V_{β} gene segment containing cosmid clones with probes from each of the 17 mouse V_{β} subfamilies. All of the mouse V_{β} probes hybridized only to the expected human V_{β} gene segments, except for the murine $V_{\beta}13$ probe, which hybridized strongly to two restriction fragments on cosmid clones H7.1 and H18.1, previously lacking known homologous human V_{β} gene segments (Fig. 1*c*). Thus, two putative new human V_{β} gene segments were identified in this study.

Comparison of human and murine V_{β} gene segments shows a striking conservation of the relative order of murine and human V_{β} homologues particularly in the 3' region of these gene families (Fig. 3*a* arrows). Recently, the physical map of the murine β -gene family has been published^{7,13}. Figure 3 shows a comparison of the physical maps of the human and murine V_{β} gene families. The solid lines connecting the two maps indicate homologous

V_{β} gene segments, as determined by hybridization to human cosmid clones with mouse V_{β} probes and/or nucleotide sequence homology. We have also assigned the position of two human V_{β} gene segments ($V_{\beta}7$, 9) based on the location of their corresponding mapped murine homologues (Fig. 3, dotted lines). If the presumed positions of the human $V_{\beta}7$ and 9 gene segments are confirmed, the relative order of V_{β} gene segments across the entire β gene locus may be conserved, apart from the internal duplications in the human β -gene locus.

The conservation of the relative orders of the V_{β} gene segments among species has at least two possible explanations. First, the evolutionary mechanisms operating on these families may have by chance been such that the order is conserved in those species which have been examined. Clearly multiple gene duplication events and, in some cases, translocations have occurred across large distances in the evolution of the human V_{β} locus. Yet despite these changes, the overall organization of the human and mouse V_{β} loci appears to be conserved. A second possibility is that some type of selective pressure may have operated to conserve the relative V_{β} -gene segment orders in these two species. Such a selective pressure could, for example, arise from the need to express sets of V_{β} gene segments at distinct developmental stages. Examples of developmental control of gene expression which correlates to chromosomal position in a multigene family are found with mammalian globin genes¹⁸, silk moth chorion genes¹⁹ and the immunoglobulin gene families²⁰⁻²².

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Interactions of sea-urchin skeleton macromolecules with growing calcite crystals—a study of intracrystalline proteins

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The exoskeletons of sea urchins are composed of magnesium-bearing calcite. Individual test plates and spines behave as single crystals in polarized light or when examined by X-ray diffraction¹⁻³. They do not, however, cleave like inorganic calcite crystals along the {104} hexagonal cleavage planes, but have conchoidal fracture surfaces reminiscent of amorphous glass. Discussion of this paradox revolves around whether the phase is monocrystalline^{2,4,5}, multicrystalline⁶, or some combination thereof⁷, but provides no explanation for the phenomenon. To address this question we grew crystals of calcite in the presence of acidic glycoproteins extracted from within the mineralized hard parts of sea-urchin tests⁸⁻¹⁰. As a control we used analogous proteins from the calcitic layer of a mollusc shell which are known to be nucleators of calcite when adsorbed on a rigid substrate, but inhibitors when in solution¹¹⁻¹³. We show that the sea urchin, but not the mollusc macromolecules selectively adsorb onto specific calcite crystal planes and with continued crystal growth are occluded inside the solid phase. These synthetic crystals fracture with a conchoidal cleavage similar to that observed in sea-urchin calcite. Thus intracrystalline proteins may be responsible for this phenomenon in biology and the manner in which they affect the mechanical properties of the crystals may also have interesting implications to the materials sciences.

Organic matrix macromolecules from the sea urchin *Paracentrotus lividus* (eastern Mediterranean) tests (SU) and from the mollusc *Mytilus californianus* (California) prismatic layer

Table 1 Planes of interaction between acidic glycoproteins and crystals, and the concentration of the proteins inside the crystals

Protein fraction	Mineral	
	Calcium maleate	Calcite
Echinoid SU glycoproteins	{110} specific 4.4 ± 4* (1.0)†; 18.7 ± 0.3 (2.0)	{110} specific 370 ± 150 (2.0); 475 (5.0); 1128 ± 370 (8.0)
Mollusc MCP glycoproteins	{110} specific 8.2 (0.5); 16.3 ± 4 (1.0); 46.5 ± 8 (2.0)	{104} non-specific 68 (1.0); 150 ± 40 (2.0)

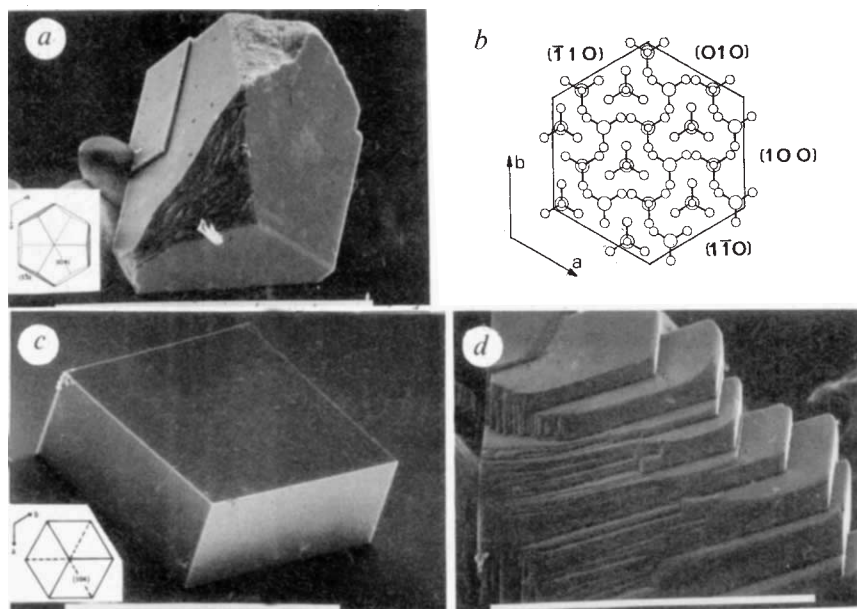
* Concentration of protein in crystals (ng protein per mg crystals) was determined by measuring the concentrations of ϵ -dansyl-lysine of labelled occluded proteins by high pressure liquid chromatography (HPLC) after hydrolysis¹⁹. The SU protein concentrations in calcite were measured by HPLC after pre-column derivatization with orthophthalaldehyde²⁰.

† Numbers in parentheses designate the concentration of protein in solution in which the crystals were grown (μ g protein per ml).

(MCP) were allowed to interact from solution with growing crystals of calcium maleate and calcite. Changes in crystal morphology as compared to controls grown in the absence of additive, identify crystal planes with which the macromolecules specifically interact^{11,14} (Table 1). The {110} faces of calcium maleate with which both SU and MCP proteins interact specifically are characterized by rows of carboxylates emerging perpendicular to the face, thus conforming to the stereochemical effect previously identified using mollusc shell proteins^{11,12}. About twice as much SU protein is required to achieve the same effect as with MCP protein. Calcite crystals grown in the presence of SU proteins in solution ($2.0 \mu\text{g ml}^{-1}$) develop a new set of faces which are heavily kinked (Fig. 1a), as is typical of unstable faces. The relative areas of these faces increases with increasing protein in solution, until their overall morphology becomes dodecahedral (at $8 \mu\text{g ml}^{-1}$). Measurement of a typical

Fig. 1 *a*, Scanning electron micrograph of a typical calcite crystal, grown in the presence of $2 \mu\text{g ml}^{-1}$ sea-urchin protein in solution. Scale bar, $100 \mu\text{m}$. The rough faces were indexed on a Nonius CAD-4 diffractometer¹⁴ as $\{3\bar{3}1\}$ faces. Insert: computer-drawn morphology of such a typical crystal, viewed down the *c* axis. *b*, Crystal structure of calcite projected on to the *ab* plane, delineated by $\{1\bar{1}0\}$ faces, expressed in the presence of SU protein. *c*, Scanning electron micrograph of a typical calcite crystal, grown in the absence of proteins. Scale bar, $100 \mu\text{m}$. Insert: computer-drawn morphology of the crystal, viewed down the *c* axis. *d*, Scanning electron micrograph of a calcite crystal grown in the presence of $2 \mu\text{g ml}^{-1}$ mollusc shell protein in solution. Scale bar, $100 \mu\text{m}$.

Methods. *a*, The proteins were extracted and fractionated following the procedure of Weiner⁸ except that instead of collagenase treatment the test plates were first cleaned in NaOCl for 30 min, washed and dried. They were then ground to a powder ($<1 \text{ mm}$ particles) sonicated for 1 min under reduced pressure in NaOCl and left on a rocking table for an additional 30 min before washing and drying. Spectrapor-3 dialysis tubing was used. Calcite crystals were grown as described¹³ on glass cover slips. *d*, Protein was extracted according to the method of Weiner¹⁶ and fractionated as described in¹⁷.



crystal on an X-ray diffractometer identified the affected faces as $\{3\bar{3}1\}$. As the *c* axis in calcite ($\sim 17 \text{ \AA}$) is much longer than the *a* axis ($\sim 5 \text{ \AA}$), the affected faces are almost parallel to the *c* axis (within $\sim 5^\circ$). The assigned indices may differ slightly from crystal to crystal and probably arise from a combination of $\{1\bar{1}0\}$ faces with the stable $\{104\}$ faces. For simplicity we treat these faces as $\{1\bar{1}0\}$. The planar carbonate molecules are perpendicular to the $\{1\bar{1}0\}$ faces, with two oxygens of each carbonate available for binding at the surface (Fig. 1*b*). Note that calcite crystals grown in the presence of MCP proteins do not develop $\{1\bar{1}0\}$ or any other unique faces, but express only the stable $\{104\}$ faces (Table 1 and ref. 11) which are the ones observed in pure calcite crystals (Fig. 1*c*). The crystals develop macrosteps when grown in solutions containing $\sim 0.5 \mu\text{g ml}^{-1}$ MCP protein, typical of non-specific inhibition of polyelectrolytes on ionic crystals (Fig. 1*d*). At $\sim 1 \mu\text{g ml}^{-1}$ the crystals lose their $\{104\}$ rhombohedral morphology and at higher protein concentrations the MCP proteins totally inhibit calcite nucleation from solution.

Calcite crystals grown in the presence of fluorescent-labelled SU (Fig. 2*a*) or MCP proteins were completely illuminated in fluorescent light (Fig. 2*b*). Partially dissolved crystals with no

remaining original surfaces also fluoresced (Fig. 2*c*), showing that some of the protein is occluded inside the crystal. The concentrations of protein inside these crystals were measured (Table 1).

The presence of specifically occluded SU protein can conceivably modify the fracture properties of calcite crystals, by continuous interference of the proteins adsorbed on $\{1\bar{1}0\}$ planes, with the $\{104\}$ natural cleavage planes. The cleavage behaviour of the synthetic crystals, pure and grown in the presence of SU or MCP proteins (for comparison) was consequently investigated. Crystals grown without protein readily cleaved along the $\{104\}$ cleavage rhombohedron planes and display sharp edges, smooth surfaces and well defined steps (Fig. 3*a*). Crystals with occluded MCP proteins are brittle and tend to readily disintegrate, with the broken surfaces revealing series of $\{104\}$ steps (Fig. 3*b*). Crystals with occluded SU proteins, on the other hand, cleave with difficulty, and their exposed fracture surfaces have a curved glassy appearance (Fig. 3*c*) reminiscent of the fracture surfaces of echinoid tests and spines (Fig. 3*d*).

The partial amino-acid composition of the SU acidic protein fraction is Asp 16, Glu 13, Gly 24, Ser 8, Ala 10 and Lys < 1 mole per cent, and the MCP fraction is Asp 30, Glu 16, Gly 13, Ser 11, Pro 7 and Lys 4 mole per cent. The SU proteins are, therefore, less acidic than the MCP proteins and Fourier-transform infrared spectra show that they also contain fewer sugar and sulphate groups. The fact that the SU glycoproteins are less acidic than the MCP glycoproteins, can account for some of the observed phenomena. The two classes of proteins interact specifically with the same surfaces of calcium maleate crystals, indicating the presence of a common stereochemical motif, but the less acidic SU proteins need to be present in higher concentrations to achieve the same effect. In contrast to calcium maleate, calcite crystals have strongly ionic surfaces and, therefore, much larger amounts of glycoproteins are occluded. The highly charged mollusc proteins interact non-specifically from solution with any calcite surface. This is consistent with the ability of the strongly ionic MCP, but not SU, proteins to nucleate calcite crystals from the (001) plane by concentrating and ordering calcium ions¹³. Significantly, the less acidic SU proteins are occluded specifically inside the calcite crystals in even larger amounts than MCP. Clearly, more detailed information on the molecular structure of these macromolecules is needed to fully understand these phenomena.

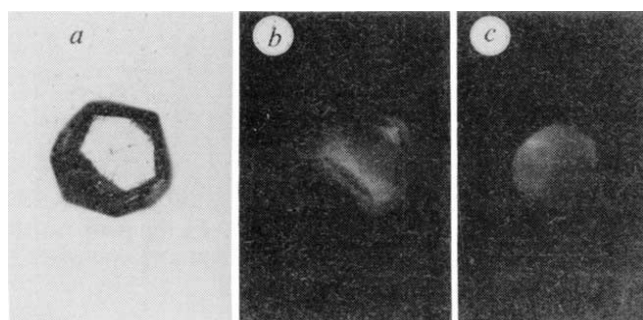
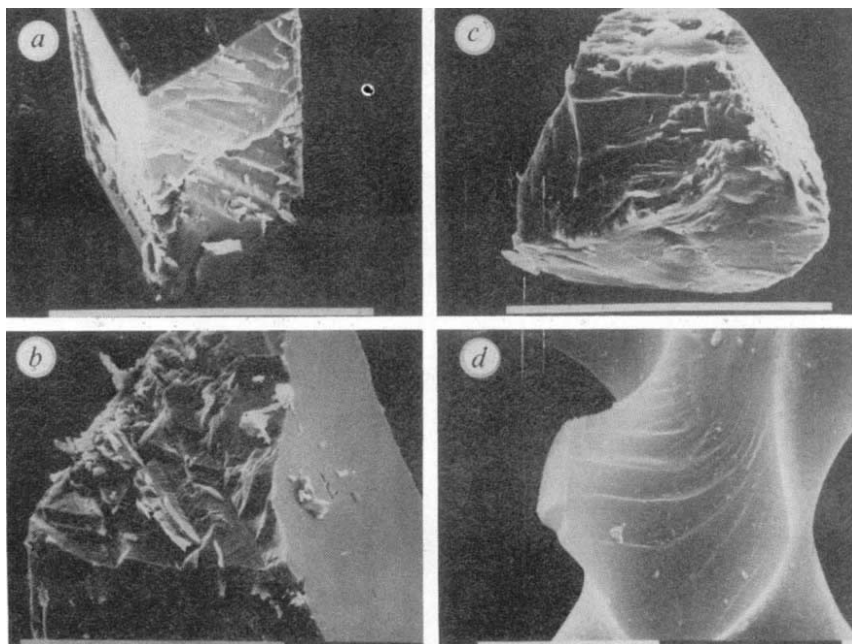


Fig. 2 *a*, Optical micrograph of a calcite crystal ($\sim 100 \mu\text{m}$), grown in the presence of $5 \mu\text{g ml}^{-1}$ fluorescently labelled SU protein. The habit is the same as that of Fig. 1*a*. Labelling was performed by dansylation of lysine residues with dansyl-chloride¹⁸. *b*, Micrograph of the fluorescence emitted from the same crystal. (Fluorescence microscope, Zeiss, dansyl filter set). *c*, Micrograph of the fluorescence emitted from the same crystal after partial dissolution.

Fig. 3 Scanning electron micrographs of cleaved calcite crystals. The crystals were cleaved under an optical microscope ($\times 50$) by touching with a razor blade and transferred to a scanning electron microscope (SEM) stub. *a*, Calcite crystal grown in the absence of protein. Note the almost smooth appearance of the $\{104\}$ cleaved face. Scale bar, $100\ \mu\text{m}$. *b*, Calcite crystal grown in the presence of mollusc shell protein ($2\ \mu\text{g ml}^{-1}$). Note the stepped nature of the cleaved face. The steps all display $\{104\}$ faces in different directions. Scale bar, $100\ \mu\text{m}$. *c*, Calcite crystal grown in the presence of sea-urchin protein ($8\ \mu\text{g ml}^{-1}$). The cleaved face shows many curved surfaces and resembles the fracture of a glassy material. Scale bar, $100\ \mu\text{m}$. *d*, Broken sea-urchin spine, showing the conchoidal nature of the broken surface. Scale bar, $10\ \mu\text{m}$.



We envisage that occlusion of sea-urchin proteins into the crystals occurs by specific adsorption followed by overgrowth of the protein, thus generating a composite material at the molecular level. The resulting effect on cleavage is conceivably due to the continuous interference of the protein adsorbed on $\{1\bar{1}0\}$ planes with the $\{104\}$ cleavage planes. Even though the *in vitro* experiments differ substantially from the *in vivo* environment, these observations provide a qualitative explanation for one of the most puzzling features of the sea-urchin mineral phase, namely, the glassy type of fracture accompanied by the unusual strength of the material¹⁵.

A rough estimate of the distribution of protein inside the calcite crystals, based on the amount of SU proteins occluded in calcite *in vitro* and on direct measurements of protein in sea urchin tests (0.02 weight per cent) and arbitrarily assuming an average relative molecular mass of 10,000, shows that only about ten or so protein molecules are present in a block of 1×10^6 unit cells. It is therefore most unlikely that these proteins form a continuous phase in the crystal. As the protein cannot be part of a perfect lattice, the site of adsorption and overgrowth must represent a discontinuity in the crystal, or in other words, the protein must be located at the surface of so called 'mosaic blocks'. O'Neill⁶ fractured sea-urchin spines after stress relaxation and observed crystal whiskers oriented along the *c* axis, with an average width of 200 unit cells. The formation of whiskers may be an indication that fracture occurs not between individual crystallites but between mosaic blocks inside a single crystal. Crystals are normally composed of mosaic blocks, and it is therefore difficult to distinguish between a single crystal with mosaic blocks and a perfectly aligned polycrystalline ensemble, in the absence of a precise knowledge of the stages of development of the mineral phase. This means that one would need to know whether or not the mature composite crystalline phase was the product of a single nucleation event, and whether it underwent recrystallization, as suggested by Towe⁷. The *in vitro* results reported here, are relevant not only to improving our understanding of echinoderm biomineralization, but suggest a novel way to engineer composite materials which have the hardness and order of crystals, but not their brittleness.

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Erratum

Large isotopic anomalies of Si, C, N and noble gases in interstellar silicon carbide from the Murray meteorite

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In this letter the acknowledgments section was omitted in the final printed version. It should read:

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